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UNIVERSITY OF ALBERTA

STUDIES ON EGG YOLK LIPIDS AND OVO-CHOLESTEROL METABOLISM

by

ZHIRONG JIANG ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

POULTRY NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1992

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This thesis is dedicated

to my parents

and all my friends in China and Canada

ABSTRACT

The two main objectives of this thesis were: 1) to examine the influence of egg yolk lipids on ovo-cholesterol metabolism; and 2) to explore the possibility of altering the cholesterolemic properties of chicken eggs by modifying the yolk fatty acid composition.

In preliminary studies on egg cholesterol values, different methods for egg cholesterol assay were compared. It was demonstrated that the conventional colorimetric method overestimated the egg cholesterol content. The egg cholesterol value was associated with the size of eggs and yolks, which in turn were related to the age of laying hens.

In rats, feeding whole yolk powder resulted in a higher ratio of high-density lipoprotein cholesterol (HDL-C) to total cholesterol (TC) and lower liver cholesterol contents when compared to the same amount of pure cholesterol, indicating that yolk components other than cholesterol per se might also play a role. Subsequent studies with yolk neutral oil (triglycerides) and yolk phospholipids (lecithin) showed that yolk neutral oil was hypocholesterolemic when compared to saturated coconut oil, and that dietary yolk lecithin resulted in a significantly higher plasma HDL-C and lower liver cholesterol contents than lard or soybean oil did.

In an attempt to decrease the cholesterolemic effects of the chicken eggs, full-fat oil seeds rich in n-6 or n-3 polyunsaturated (PUFA) or n-9 monounsaturated fatty acids (MUFA) were included in laying hen rations to enrich the eggs with PUFA or MUFA. Feeding flax seeds to laying hens resulted in the accumulation of α-linolenic acid and its longer chain metabolites, such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) in the egg yolk total and phospholipids. Feeding the n-3 PUFA enriched eggs to rats reduced plasma and liver cholesterol contents, enriched plasma and tissue total and phospholipids with n-3 PUFA, and inhibited the synthesis of prostaglandin E_2 by the skeletal muscle when compared to the control or n-6 PUFA enriched eggs. In healthy human volunteers, consumption of two n-3 PUFA eggs a day for a period of 18 days prevented plasma TC and LDL-C from rising, increased HDL-C, and reduced plasma triglyceride level. The overall qualities of the n-3 eggs were not adversely affected. A slight off-flavor, however, was detected in some of these eggs, indicating the need for further research work.

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Chapter 1. General Introduction

The chicken egg, viewed by many as one of nature's most perfect foods, has been a staple food item for humans since the dawn of civilization. Domestication of chickens to supply egg and meat dates back to 1400 BC in China and Egypt (Moreng and Avens, 1985). The egg has long been recognized as a rich source of essential nutrients for maintaining human health. It provides the most complete, therefore the highest quality proteins, all necessary vitamins except vitamin C, and minerals (Moreng and Avens, 1985; Shrimpton, 1987).

The per capita consumption of the egg, however, has been declining steadily over the past four decades in Western countries (Figure 1.1). Changing life style and more choices of food products are two contributing factors. But by far the major cause is the consumers' increasing health consciousness of diet and the controversial relationship between dietary cholesterol and coronary heart disease (CHD). Due to its relatively high cholesterol level and culinary advantage, the egg has been used as the exclusive source of dietary cholesterol in both animal and human experiments to study the effect of dietary cholesterol. Consequently, the term "egg" today has become the synonym for "cholesterol" which, in turn, implicates heart disease, to many laymen and medical professionals alike, although the relationship between dietary cholesterol and CHD is far from being settled even today.

1.1. About Cholesterol

The compound now known as cholesterol was first described by a

French chemist de Fourcroy, who in 1789 isolated a crystalline substance from the alcohol-soluble fraction of human gallstones (de Fourcroy, 1789). By the early nineteenth century, the newly discovered compound was found in human and animal bile (Chevreul, 1816), and in human blood (Lecanu, 1838). The universal distribution of the compound in all animal cells was soon recognized. The compound was found to be an alcohol by Berthelot in 1859 (Berthelot, 1859) and its correct empirical formula, $C_{27}H_{46}O$, was reported by Reinitzer in 1888 (Reinitzer, 1888). The final structure of cholesterol (Figure 1.2) was established in 1932 by Windaus (1932) and Wieland and Dane (1932).

1.1.1. Distribution of Cholesterol

Cholesterol is almost ubiquitously distributed in cells of all animals. In mammals, the highest concentration of cholesterol is found in the adrenal and brain tissues (Sabine, 1977; Gibbons et al., 1982). The proximate distribution of cholesterol in human beings is shown in Table 1.1. The largest absolute amounts of cholesterol are contained in muscle, brain and nervous system, and connective tissues including adipose. Cholesterol can be present predominantly in the free or esterified forms, with a small proportion being sulfated or conjugated (Sabine, 1977). In virtually all cells, except adipose tissue, the bulk of cellular cholesterol is contained in or associated with cellular membranes. Cells of most other tissues have similar patterns of cellular cholesterol distribution, except in some like neurons where the myelin sheath contains more than 70% of cellular cholesterol (Davison, 1970).

1.1.2. Physiological Functions of Cholesterol

Cholesterol is an important precursor to a variety of key biological substances. It is converted in the liver into bile acids which are indispensable for the digestion and absorption of dietary lipids (Stryer, 1981). A whole array of steroid hormones such as progesterone, estrogens, androgens, glucocorticoids, cortisone, hydrocortisone, and aldosterone are synthesized from cholesterol and are essential for normal sexual function, anti-inflammation, and sodium-regulation. In the skin, a precursor of cholesterol, 7-dehydro-cholesterol, is converted to vitamin D_3 necessary for homeostasis of calcium-phosphate metabolism. Some precursor molecules of cholesterol are also necessary for the de novo synthesis of biologically important compounds such as ubiquinones and dolichol (Health and Welfare Canada, 1990). An essential compound of biological membranes, cholesterol influences membrane fluidity. Consequently, membrane permeability to water and solutes, membrane fusion, and activities of membrane-bound enzymes, hormone and other receptors are altered by the presence of cholesterol (Sabine, 1977; Bloch, 1985). One of the most intriguing facts about cholesterol is its ubiquitous distribution in animals but not in plants in which other sterols are present. Cholesterol plays a unique role in the transport of fats which serve as the energy reserves in animals in the form of lipoproteins. In plants, carbohydrates serve as the energy reserves which are transported in water-soluble form. This biological difference may explain the animal origin of cholesterol (Gibbons et al., 1982), although this division may no longer be accurate (Bloch, 1985).

1.1.3. Biosynthesis of Cholesterol and Its Regulation

Not only is cholesterol ubiquitously distributed, it is also synthesized de novo in cells of virtually all animals (Bloch, 1985). The detailed biosynthetic pathway leading the formation of cholesterol in animals is now clear owing to the work of Bloch, Cornforth, and Popjak and their respective colleagues. Most of more than 30 enzymes of cholesterogenesis are membrane bound. The synthetic pathway conveniently divided into four major stages is presented briefly as follows (Fielding and Fielding, 1985):

1. Conversion of acetyl CoA into mevalonate. Three acetyl CoA molecules are condensed to form 3-hydroxy-3-methylglutaryl CoA (HMG CoA) which is then reduced to mevalonic acid by HMG CoA reductase in the presence of NADPH. The reduction of HMG CoA by HMG CoA reductase, a transmembrane protein with a molecular weight of 97 K, is a rate limiting step in the de novo synthesis of cholesterol.
2. Mevalonate to squalene. Mevalonic acid is subsequently phosphorylated and decarboxylated to form isopentenyl pyrophosphate. Then six units of isopentenyl pyrophosphate are condensed to form presqualene pyrophosphate, which is converted in the presence of NADPH to squalene.
3. Squalene to lanosterol. Squalene is first converted to squalene-2,3-oxide by squalene epoxidase in the presence of NADPH, FAD, and oxygen. Squalene-2,3-oxide is then cyclized by the microsome 2,3-oxidosqualene:lanosterol cyclase to form lanosterol.
4. From lanosterol to cholesterol. The conversion of lanosterol to cholesterol, catalysed by microsome enzymes, involves 19-steps of

reaction including demethylation, reduction, and rearrangement of double bonds.

As mentioned above, the conversion of HMG CoA to mevalonic acid is the committed step in the sequence of reactions leading to the de novo synthesis of cholesterol. As would be expected for a rate-limiting enzyme, the activity of HMG CoA reductase is highly regulated. For instance, rat hepatic HMG CoA reductase activity is at least 4-fold higher at mid-dark than that at mid-light (Edwards et al., 1972). This diurnal change is attributed to a lower cellular free cholesterol concentration caused by the conversion of cholesterol to bile acids which are necessary for the digestion and absorption of food (Edwards et al., 1972). Two kinds of regulatory mechanisms of the HMG CoA reductase activity have been proposed. The first involves the stringent control of the rate of reductase protein synthesis (Rodwell et al., 1976). A wide variety of oxygenated cholesterol derivatives, not cholesterol per se, are reported to inhibit cholesterogenesis probably by lowering the level of cellular HMG CoA reductase (Shroepfer 1981; Tanaka et al., 1983; Sexton et al., 1983). HMG CoA reductase is also regulated by reversible phosphorylation mediated by a phosphate-kinase cascade (Hunter and Rodwell, 1980). The majority of the reductase in vertebrate liver may be present in a catalytically inactive form in vivo (Hunter and Rodwell, 1980). The major sites of de novo cholesterol synthesis are the liver and the intestine. In monkeys, the liver and intestine contribute about 44% and 23%, respectively, of the total cholesterol synthesis in the whole body (Spady and Dietschy, 1983).

1.1.4. Metabolism of Cholesterol

Another major source of body cholesterol is diet. In Canada, for instance, the average intake of cholesterol is about 440 mg per day per person (Health and Welfare Canada, 1990). Only a small proportion of dietary cholesterol is esterified and the cholesterol esters are hydrolysed before absorption. The exogenous free cholesterol of dietary origin mixes freely in the lumen of the small intestine with endogenous cholesterol of biliary origin, and the free cholesterol is incorporated into mixed micelles containing phospholipids, conjugated bile acids, monoglycerides, and free fatty acids (Dietschy, 1978). The absorption of cholesterol along with monoglycerides, fatty acids and phospholipids occurs mainly in the jejunum. It has been speculated that the close association of micelle with plasma membranes of enterocytes promotes the transfer of cholesterol from micelle into the lipid bilayer of mucosa cell membrane and thus entering the mucosa cells (Moore, 1987). The efficiency of absorption of dietary cholesterol varies widely among individuals and averages about 37% irrespective of dietary cholesterol levels in the range of 400 to 1200 mg/day (Kudchodkar, 1973; Connor and Lin, 1974; Whyte et al., 1977). The absorption of dietary cholesterol is inhibited by plant sterols such as ß-sitosterol (Grundy and Mok, 1976, 1977) and increased by the addition of fat (Treadwell and Vahouny, 1968). De novo synthesis and absorption of cholesterol in the mucosa cells of the intestine also are regulated by the ingestion of cholesterol and certain fats as they are in cells of other organs (Field et al., 1990).

Cholesterol of both endogenous and exogenous origin in the liver and intestine is secreted into circulating plasma in the form of lipoproteins. Lipoproteins are multimolecular complexes which can be classified on the basis of lipid or protein compositions or physical properties (Gibbons et al., 1982). Most commonly, they are classified on the basis of their flotation density. The lipoproteins of normal human plasma and their characteristics are presented in Table 1.2. In other mammals and birds, lipoproteins of similar chemical composition and physical properties are also present in the plasma, even though the density range of a given lipoprotein and the proportion of lipoproteins in animal plasma may differ from those of in human plasma (Soutar and Myant, 1979). The metabolic fates of major plasma lipoproteins are briefly discussed as follows.

1. Chylomicrons. These triacylglycerol-rich lipoproteins are synthesized and secreted by the intestine during digestion and absorption of dietary lipids. Free cholesterol, free fatty acids, and monoglycerides are re-esterified to form cholesterol esters and triacylglycerols in the mucosa cells, and then secreted into intestinal lymph in a complex with apolipoprotein B-48 and phospholipids (Fielding and Fielding, 1985). During circulation, most of the chylomicron triacylglycerols (80%) are hydrolysed by lipoprotein lipase at the endothelial surface of the vascular bed (Cryer 1983). The remnants are cleared by a specific, high-affinity receptor in the liver which recognized apo E (Windler et al., 1980; Mahley and Innerarity, 1983).

2. Very-low density lipoproteins (VLDL) and low-density lipoproteins (LDL). Another triacylglycerol-rich lipoprotein is VLDL secreted by the liver. Cellular cholesterol, phospholipids, triacylglycerols synthesized in the smooth endoplasmic reticulum, and apoprotein B-100 synthesized in

the rough endoplasmic reticulum are assembled probably in the Golgi apparatus and then secreted directly into plasma (Fielding and Fielding, 1985). They acquire cholesterol esters from high-density lipoproteins in exchange for triacylglycerols, which is mediated by cholesterol ester exchange protein (Zilversmith et al., 1975). VLDL undergo lipolysis of triacylglycerols by lipoprotein lipase at the surface of capillary endothelial cells, leaving residual particles called VLDL remnants (Grundy, 1986). A significant fraction of VLDL remnants are removed by the liver via either chylomicron remnant receptors (apo E receptor) or LDL receptors (apo B, E receptor) (Goldstein and Brown, 1982; Stalenhoef et al., 1984). In humans, 50 to 60% of the VLDL remnant particles are cleared by this direct pathway (Egusa et al., 1985; Kesaniemi et al., 1985) while the rest are transformed into LDL. At present, the complex steps involved in the conversion of VLDL remnants into LDL are not fully understood. It has been suggested that the transformation requires the attachment of VLDL remnants to hepatic apo E receptors (Grundy, 1986).

The major site of LDL removal is the liver. Up to 75 to 85% of LDL is removed by the liver from the circulation (Grundy, 1986). The major pathway for the clearance of LDL is via LDL receptor, while lesser amounts of LDL are removed by nonspecific pathways. When LDL or VLDL remnants bind to LDL receptors, the resulting lipoprotein-receptor complexes are internalized to form endocytic vesicles which are fused with lysosomes (Innerarity et al., 1980). While LDL receptors are recycled to the plasma membrane to form new receptors, the LDL particles are degraded. Cholesterol ester is hydrolysed to contribute to the cellular free cholesterol pool (Brown and Goldstein, 1983).

3. High-density lipoproteins (HDL). The HDL represent a complex group of particles which are derived either through the secretion by liver and small intestine or from the lipolyses of the chylomicrons or VLDL (Tall and Small, 1980). The principle apolipoproteins of HDL are apo A-I and apo A-II, which constitute more than 90% of total HDL apoprotein. But HDL also contains small fractions of apo C, D, E and F (Tall and Small, 1980). Apo A-I is synthesized by the intestine as part of chylomicrons and is released to HDL during the lipolysis of chylomicrons (Grundy, 1986). Both apo A-I and apo A-II are secreted by liver in association with nascent HDL. HDL are thought by many to play a major role in the transport of cholesterol from extrahepatic tissues where cholesterol is not actively degraded back to the liver where the chief pathway of cholesterol catabolism (bile secretion) occurs (Grundy, 1986). Free cholesterol released from plasma membranes is esterified via lecithin:cholesterol acyl transferase (LCAT) and then deposited into nascent or smaller HDL (Fielding and Fielding, 1982). Some of the cholesterol esters of HDL is transferred to other lipoproteins, such as VLDL, VLDL remnants and LDL (Nestel et al, 1979). HDL are removed mainly by the liver possibly via apo A receptors (Miller et al, 1977; Oram et al., 1983) and other organs (Carew et al., 1976).

The liver is thus the major organ responsible for the removal of circulating chylomicron and VLDL remnants, LDL and HDL particles, all of which are rich in cholesterol. The major routes of cholesterol removal from the liver are 1) secretion of biliary cholesterol; and 2) conversion of cholesterol into bile acids. In human adults about 1 gram of cholesterol leaves the liver each day as biliary cholesterol (Grundy and

Metzger, 1972). The rate of cholesterol secretion into bile is a function of the output of bile acids (Hardison and Apter, 1972; Wheeler and King, 1972). Two primary bile acids produced in the liver are cholic and chenodeoxycholic acids. The detailed metabolic pathway involved in bile acid synthesis from cholesterol has been fully elucidated (Danielsson and Einarsson, 1969). The initial reaction is the conversion of cholesterol into 7-α-hydroxycholesterol catalysed by a microsome 7-α-hydroxylase. This step is rate-limiting in the synthesis of bile acids (Myant and Mitropoulos, 1977). The bile acids synthesized are conjugated with either glycine or taurine to enhance the detergent properties of the bile acids. In man, the conjugated bile acids are secreted with cholesterol and phospholipids into the duodenal lumen via the common bile duct. They play an essential part in the digestion and absorption of cholesterol and other dietary lipids in the proximal region of the small intestine. The bile acids are subsequently reabsorbed, mainly by an active transport mechanism, from the distal region of the ileum. They enter the portal circulation and are extracted almost completely in their first pass through the liver. Under normal circumstances, about 95 to 98% of bile acids entering the intestine are reabsorbed. After appropriate modifications, bile acids are secreted into bile and returned to the duodenal lumen, thus completing the so-called enterohepatic cycle of the bile acids (Grundy, 1986). Bile acids and cholesterol which are not reabsorbed in the enterohepatic circulation are excreted into the feces, which is the only major pathway for cholesterol elimination from the animal body.

1.2. Atherosclerosis and the Role of Cholesterol

Cardiovascular disease remains the number one cause of death in Canada (Health and Welfare Canada, 1990). In the U.S., slightly less than one-half of the men and women die from a degenerative vascular disease related to atherosclerosis (Castelli et al., 1990). One of the unifying theories of the pathogenesis of atherosclerosis and the role of plasma and lipoprotein lipids will be briefly discussed.

In experimental atherogenesis models, the arterial lesion-prone or prelesion areas can be delineated by their in vivo uptake of the protein-binding dye, Evans Blue, before the plaques become visible macroscopically or microscopically (McGill et al., 1957; Fry, 1973). Salient features of prelesion areas include an increased endothelial permeability to and intimal accumulation of plasma proteins including albumin, fibrinogen, and low-density lipoprotein (apo B) (Schwartz et al., 1991). The intimal cholesterol retention and monocyte recruitment are also markedly increased (Caplan and Schwartz, 1973). Thus, the initial events in atherogenesis include an enhanced endothelial transcytosis of plasma proteins including LDL particles and monocytes, the two key participants in atherogenesis. The uptake of both components is markedly augmented in the presence of hyper-cholesterolemia, hyperlipidemia, and oxidatively modified LDL particles (Schwartz et al., 1991).

The monocytes then migrate through endothelium into the subendothelial space (SES) in response chemoattractants such as monocyte chemotactic protein-1 (MCP-1) (Yoshimura et al., 1989). The MCP-1 is synthesized by both smooth muscle cells and endothelial cells and the secretion is augmented by the presence of minimally modified (oxidized)

LDL (Cushing et al., 1990). Another monocyte chemoattractant is the oxidized LDL particles per se (Quinn et al., 1987). Within the SES, the monocytes are activated and differentiate into macrophages (Schwartz et al., 1991). This process modulates the phenotypic expression of the scavenger receptors (Goldstein et al., 1979). The scavenger or acetylated LDL receptors bind not with natural LDL particles, but preferentially with modified LDL such as oxidized and malondialdehyde modified LDL (Brown et al., 1980; Haberland et al., 1984; Henricksen et al., 1981; Steinbrecher et al., 1984; Parthasarathy, 1987; Fogelman et al., 1980). Unlike the apo B, E receptors, the scavenger receptors are not down-regulated with the intracellular accumulation of cholesterol (Schwartz et al., 1991). The macrophages are thus transformed into cholesterol ester-rich foam cells through the non-down-regulated uptaking of oxidized LDL via scavenger receptors (Fogelman et al., 1980). As long as the plasma LDL level is elevated, the oxidation potential of intimal LDL is high, and the oxidized LDL is relentlessly taken up by the scavenger receptors, foam cells will continue to form and accumulate to generate the fatty streak (Schwartz, 1991). Until this stage, lipids accumulated are predominantly intracellular and foam cells show little or no evidence of necrosis. If plasma LDL and oxidation of intimal LDL are reduced and the HDL-mediated reverse cholesterol transport is facilitated, regression of foam cells and fatty streaks may occur.

If atherogenesis progresses, however, the foam cells of fatty streaks are injured from overloading with the oxidized LDL particles and will disrupt. This cellular necrosis releases intracellular lipids to the interstitium, giving rise to the cholesterol-ester rich core of the atherosclerotic plaque. It also induces the mitogen-stimulated proliferation of smooth muscle cells (Benditt and Benditt, 1973), and the subsequent synthesis of collagens, elastin, and proteoglycans (Schwartz et al., 1991). A triad of changes follows, namely neovascularization, fibrosis, and lymphocytosis which indicates the involvement of an autoimmune process (Schwartz and Mitchell, 1962; Schwartz et al., 1989; Schwartz et al., 1990) leading to the formation of mature or fibrous atherosclerotic plaque. Human plaque apo B has been recently shown to be immunogenic (Hollander et al., 1990). The arterial lumen is narrowed with the established plaque. Arterial occlusion occurs as the result of thrombosis at severely narrowed sites when the mature plaque ruptures, exposing extracellular lipids, collagens, and other microfibrillar materials to the circulating platelets (Schwartz, 1991).

It is clear from discussions above that plasma and lipoprotein lipids play some pivotal role in atherogenesis. Elevated plasma levels of cholesterol, particularly LDL cholesterol, and triacylglycerols are actively involved in the initiation, foam cells and fatty streak formation, plaque maturation, and arterial occlusion. An active reverse cholesterol transport mediated by HDL particles is of great importance for the prevention and regression of atherosclerotic plaque.

1.3. Relationship Between Dietary Cholesterol and Plasma and Lipoprotein Cholesterol Levels and Atherosclerosis

In the past 50 years in industrialized countries, there has been a pattern shift of morbidity and mortality from acute infectious diseases to chronic degenerative diseases (Health and Welfare Canada, 1990). Lifestyle, including diet habit, of the public has never before been influenced by consumers' health consciousness as much as it is today. There is a widely held view that lifestyle, including diet, plays a major role in causation of chronic diseases such as coronary heart disease (CHD). Epidemiological studies have, indeed, demonstrated that environmental factors such as diet, are more important than genetic factors in determining susceptibility to CHD (Anon, 1976). The occurring rate of CHD within one generation is also not inevitable and predetermined but is mainly affected by changes of lifestyle, especially the diet, within the country (Uemura and Pisa, 1985; Goto and Moriguchi, 1990).

1.3.1. The Lipid-Heart Hypothesis

Only a few studies directly examined the link between diet and the incidence of CHD due to the complexity of the disease per se. Most studies investigated the influence of diet on plasma total cholesterol level, and more recently, the lipoprotein cholesterol levels, assuming that plasma and lipoprotein cholesterol concentrations are associated so closely with atherosclerosis that the risk of developing CHD can be predicted from measuring their concentrations. Numerous epidemiological and intervention studies have consistently and unanimously demonstrated that an elevated plasma cholesterol (and more accurately LDL cholesterol) is atherogenic while a high HDL cholesterol is associated with low incidence of CHD (Dawber, 1980; LaRose et al., 1990; Health and Welfare Canada, 1990). This assumption, often referred to as the "lipid hypothesis", is also strongly supported by the roles that lipoproteins play in the pathogenesis of atherosclerosis as discussed in section 1.2 of this chapter. This hypothesis, however, is not without its criticism (Texon, 1989).

The influence of dietary cholesterol on plasma and lipoprotein cholesterol levels and CHD risk has been studied extensively over the last four decades. This relationship has been examined in experimental animals, in observational epidemiological studies ranging from multinational comparisons to much smaller groups with distinct eating habits, and in clinical studies using outpatients consuming various experimental diets or patients confined to metabolic wards.

1.3.2. Experimental Animal Studies

A possible relation between dietary cholesterol and atherosclerosis was first brought to light in laboratory animals. Antischkow and Chalatow (1913) showed that feeding cholesterol to rabbits caused atherosclerosis. Since then, dietary cholesterol has been demonstrated to be the primary essential agent for producing experimental atherosclerosis in various species (Sabine, 1977; Wissler and Vesselinovitch, 1987). There is, however, a wide variation in the susceptibility of laboratory animals to diet-induced atherosclerosis. For instance, the rat, mouse, and hamster are quite resistant to cholesterol-induced atherosclerosis, whereas the dog, guinea pig, and pig are moderately susceptible, and the rabbit and chicken highly susceptible (Sabine, 1977). The production of atherosclerosis by dietary cholesterol normally results in a concomitant rise in plasma cholesterol level. Recently, primate models that are phylogenetically close to humans and have lipoproteins similar to those of humans have been widely used (Strong, 1976; McGill et al., 1981; Rudel et al., 1985; Wissler and Vesselinovitch, 1987). In one study with rhesus monkeys, dietary cholesterol was used to develop two stages of severity of atherosclerosis which are comparable to atherosclerosis of 35- and 65-y old men, respectively (Clarkson et al., 1979; Wagner et al., 1980; Clarkson et al., 1984). Once they reached appropriate stages, the monkeys were fed diets either high or low in cholesterol. After four years of dietary treatment, monkeys on high cholesterol diets showed slight regression in coronary lesions, while those on low cholesterol diet had considerable regression in atherosclerotic plaques, indicating an important role of dietary cholesterol in the progression and regression of atherosclerotic plaque. It should be noted, however, that in experimental atherosclerosis, the magnitude of changes in the diet is almost always drastic and the atherosclerotic syndrome produced even in non-human primates is never identical, although similar, to that found in humans (Sabine, 1977).

1.3.3. Observational Epidemiological Studies

When examined in epidemiological and clinical studies, however, the relationship between dietary cholesterol and plasma or lipoprotein cholesterol and incidence of CHD is still as controversial today as it was 40 years ago (Oster, 1982; Connor, 1982; Stehbens, 1989; Steinberg, 1989). Several cross-sectional population studies have failed to show statistically significant correlations between dietary cholesterol and plasma cholesterol level. These include the Framingham Study (1970), the Ni-Hon-San study comparing Japanese men living in Japan, Hawaii, and California (Kato et al., 1973); and the Lipid Research Council (LRC)

Prevalence Study (Gordon et al., 1982b; Glueck et al., 1982). In the LRC Prevalence Study of a large group of white children age 6-19 y, dietary cholesterol intake failed to show significant correlations to plasma total or LDL cholesterol (Glueck et al., 1982). Other cross-populations studies, however, demonstrated a positive relationship between cholesterol intake and CHD risks (Stamler and Shekelle, 1988). In the Puerto Rico Heart Health Programme, a statistically significant positive association was found between dietary cholesterol and plasma cholesterol in urban men but not in rural men (Garcia-Palmieri et al., 1980). A statistical significant association was also demonstrated in the male population of the Western Electric Study between cholesterol intake and plasma cholesterol and CHD risk (Shekelle et al., 1981). In both studies, however, the correlation between dietary cholesterol and plasma cholesterol or death from CHD was of low order in magnitude. For instance, in the Western Electric Study, the multiple logistic coefficient between dietary cholesterol and death from CHD was only .0032, ten times less than the coefficients between dietary saturated fat or smoking and mortality from CHD (Stamler and Shekelle, 1988).

Several within-population studies have also demonstrated a positive relationship between dietary cholesterol and plasma or LDL cholesterol and CHD risk (Shekelle et al., 1981; Gordon et al., 1982a; Kroomhout et al., 1985; Kuschi et al., 1985) whereas others failed to demonstrate the association (Gordon et al., 1981; Dawber et al., 1982). In several of these studies, a positive relationship between cholesterol intake and HDL cholesterol was also demonstrated (Glueck et al., 1982; Knuiman et al., 1983) although it seems unlikely that dietary cholesterol would be protective against atherosclerosis.

A striking feature of the results obtained in the epidemiological studies is the absence of a strong, independent association between dietary cholesterol and plasma cholesterol or lipoprotein cholesterol levels or the mortality rates from CHD in larger group comparisons, and the low or zero correlation coefficients in individual studies.

1.3.4. Clinical Studies

Extensive clinical studies on the influence of dietary cholesterol generated results as divided as those from the epidemiological studies. Studies reported before 1979 were extensively reviewed in an excellent article by McGill (1979) and thus no attempt was made to discuss all the studies here. In most studies of outpatients consuming various experimental diets, dietary cholesterol has little or no effect on plasma total cholesterol levels (McGill, 1979; Grundy and Denke, 1990). On the other hand, metabolic ward studies using confined patients or prisoners have consistently demonstrated that dietary cholesterol increases plasma cholesterol (Beveridge et al., 1960; Connor et al., 1964). The quantitative relation between dietary cholesterol and plasma cholesterol levels, however, remains a matter of dispute (Keys, 1984; Hegsted, 1986). According to Keys et al. (1965), the plasma cholesterol change is the function of the square root of dietary cholesterol (Equation 1.1), whereas Hegsted et al. (1965) reported a linear relationship (Equation 1.2).

$$\Delta \text{ Chol} = 1.5\ (X_2^{1/2} - X_1^{1/2}) \qquad \text{----} \qquad \text{Equation 1.1}$$

$$\Delta \text{ Chol} = 0.176\ (X_2 - X_1) \qquad \text{----} \qquad \text{Equation 1.2}$$

where Δ Chol is the change of plasma cholesterol level in mg/dL; X_1 and X_2 the cholesterol intake as mg per 1000 kcal per day before and after the experiment, respectively.

It is also not clear in what range dietary cholesterol affects plasma cholesterol level. Connor (1970) stated that dietary cholesterol up to 110 mg/day did not influence plasma cholesterol, and beyond 300 mg/day, the increase of plasma cholesterol was small with increasing cholesterol intake.

As the roles that lipoproteins play in atherogenesis are recognized, more recent studies tend to examine the relationship between dietary cholesterol and lipoprotein cholesterol levels in addition to plasma total cholesterol (TC). Some of the recent studies will be reviewed below in chronological order.

Lin and Connor (1980) fed 1000 mg/d cholesterol to both a hypercholesterolemic and a normal subject for a period of 11 wk. Plasma TC, LDL-C, and HDL-C increased in both subjects. While the LDL-C/HDL-C ratio increased significantly in hypercholesterolemic but not in normal subjects. Serum triacylglycerol (TG) level was lowered in both groups.

Nestel et al. (1982) compared serum lipoprotein profile in six men consuming diets providing 200 or 1700 mg cholesterol/d. In four wk, plasma TC and LDL-C did not change significantly, but HDL-C increased significantly.

Schonfeld et al. (1982) found that in 20 young men, plasma LDL-C and HDL-C increased after consuming three to six eggs a day for four to six wk when dietary fat was saturated but not when dietary fat was polyunsaturated. The authers suggested that in terms of CHD risk, any rise in LDL-C upon egg consumption would be neutralized by the elevation of HDL-C.

Applebaum-Bowden et al. (1984) fed nine adults in a crossover trial on a diet with either 137 or 1034 mg of cholesterol daily. There was a significant increase of LDL-C, although TC was not significantly elevated.

Sacks et al. (1984) fed one extra large egg or one placebo per day to 17 lactovegetarians for three wk in a randomized cross-over study. LDL-C was increased but was not statistically significant while VLDL-C decreased.

Beynen and Katan (1985) reported that adding six eggs per day to an originally low cholesterol diet of health volunteers for ten days increased plasma TC (13%), LDL-C (21%), and HDL$_2$-C (35%). Consequently LDL-C/HDL$_2$-C ratio was decreased upon egg consumption. The plasma TG level was also lowered.

Oh and Miller (1985) fed three eggs per day to 21 healthy middle aged men for 28 days. Serum TC increased significantly in eight subjects (hyper-responders) but not in the others (hypo-responders). When examined in terms of LDL-C/HDL-C ratio, however, a reduction was found in both groups. Continuous egg consumption led the LDL-C/HDL-C ratio return to pre-experimental base line value.

Edington et al. (1987) reported a randomized cross over study using 168 subjects to determine whether halving or doubling the present dietary cholesterol intake (4 eggs/wk) affected blood cholesterol levels. A small but significant increase of plasma TC was found after 4 wk in the group eating 7 eggs per wk when compared with those eating 2 eggs per wk. But this difference was no longer apparent after 8 wk, indicating an adaptive response of serum cholesterol to dietary cholesterol intake.

Vorster et al. (1987) reported that in 25 rural African Hack males who habitually consumed 1240 mg cholesterol (5 to 6 eggs) per day with fat supplying 20% of total energy, serum TC was slightly but significantly higher and serum TG significantly lower than those in the control group. There was, however, a rise in HDL-C, resulting in similar HDL-C/TC ratio in both groups.

These recent studies demonstrated that egg consumption generally resulted in slight or no changes in serum TC, and significant elevations of LDL-C and HDL-C levels. The HDL-C/TC or HDL-C/LDL-C ratio was often increased, and serum TG levels reduced. The individual response to dietary cholesterol is highly variable and adaptable. Cholesterol intake (which now averages 450 mg/d in Canada and USA) is the least important of the dietary variables (see discussions herein) influencing plasma and lipoprotein lipid concentrations in healthy humans.

1.4. Other Dietary Components Affecting Plasma and Lipoprotein Cholesterol Levels and Atherosclerosis

It is clear from the discussions above that dietary cholesterol at the typical range of intake exhibits little, if any, effects on plasma or lipoprotein cholesterol concentrations or atherosclerotic risks in healthy humans. A whole array of dietary components other than cholesterol have major influences on plasma and lipoprotein cholesterol levels and atherosclerosis. These variables include 1) the quantity and quality of dietary fat; 2) carbohydrates including fibre; 3) the type of proteins;

and 4) alcohol consumption.

1.4.1. Dietary Fat

Both epidemiological and clinical studies have consistently demonstrated that the type and the quantity of dietary fat strongly affect plasma and lipoprotein cholesterol levels and atherosclerotic risk. Early works by Ahrens et al. (1957), Keys et al. (1965), and Hegsted et al. (1965) demonstrated that dietary saturated fats increase plasma cholesterol levels. Unlike in dietary cholesterol studies, these results have not been contradicted by subsequent studies. Recent studies (Mattson and Grundy, 1985; Bonanome and Grundy, 1988; Grundy and Vega, 1988) indicate that LDL-C as well as total cholesterol concentrations are raised by saturated fats in the diet. The elevation of LDL-C by saturated fat intake is suggested to be the result of a retarded receptor-mediated clearance of LDL particles (Grundy and Denke, 1990). A reduction of mRNA for LDL receptors was reported in baboons upon feeding of saturated fat (Fox et al., 1987). It has also been postulated that the enrichment of cellular phospholipids with saturated fatty acids might interfere with the normal function of LDL receptors, thus reducing the receptor-mediated clearance of LDL (Spady and Dietschy, 1988). Not all saturated fatty acids, however, have similar hypercholesterolemic effects. Medium chain fatty acids (C8:0 and C10:0) do not raise serum cholesterol (Hashim et al., 1960). Recent studies indicated that stearic acid (C18:0) does not elevate serum total cholesterol (Bonanome and Grundy, 1988), probably due to the ready conversion of stearic acid into oleic acid in the liver (Elovson, 1965). The principal saturated fatty acid in food, palmitic acid

(C16:0), is the major saturate to increase LDL-C as well as TC (Mattson and Grundy, 1985; Bonanome and Grundy, 1988). Myristic acid (C14:0), a minor component of dietary fat, also exhibits hyper-cholesterolemic action (Hegsted et al., 1965).

There are two families of polyunsaturated fatty acids (PUFA) in the diet, namely n-6 and n-3 polyunsaturates. The predominant n-6 PUFA is linoleic acid (C18:2, LA) from oilseeds. The parent n-3 PUFA is α-linolenic acid (C18:3, LNA), which is abundant in flax seeds (linseeds), canola seeds and green leaf vegetables. The longer chain n-3 PUFA, such as eicosapentaenoic acid (C20:5, EPA), and docosahexaenoic acid (C22:6, DHA) are found in marine products and animal tissues.

For many years, the so-called cholesterol-lowering polyunsaturated fatty acids were limited largely to LA. Early papers by Kinsell et al. (1953) and others (Keys et al., 1965; Hegsted et al., 1965) suggested that LA lowers serum cholesterol level about half as much as saturated fatty acids raise it. More recent work showed that when LA replaces saturated fatty acids in the diet, the major cholesterol lowering occurs in the LDL fraction (Vega et al., 1982). The proposed mechanisms of cholesterol-lowering by dietary LA include: 1) enhanced excretion of cholesterol through bile as bile acids and cholesterol (Paul et al., 1980); 2) inhibition of hepatic synthesis of apo B-containing lipoproteins such as VLDL (Cortese et al., 1983); and 3) an increase in LDL-receptor mediated clearance (Kovanen et al., 1981). In many studies, dietary LA also induced a reduction of HDL-C (Shepherd et al., 1978; Vega et al., 1982; Jackson et al., 1984; Mattson and Grundy, 1985), a non-desirable effect in terms of CHD risks.

The past decades witnessed the tremendous growth of interests and knowledge of another family of PUFA, the n-3 PUFA. Although being demonstrated a long time ago that dietary LNA and fish oil are as effective as LA in lowering cholesterol level (Bronte-Stewart et al., 1956; Sinclair, 1956; Worne and Smith, 1959; Nelson, 1972), their importance in the control of atherosclerosis was neglected until 1970s (Bang and Dyerberg, 1972; Bang et al., 1976). The beneficial effects of dietary n-3 PUFA include reducing plasma TG, TC, LDL-C, and increasing HDL-C; decreasing platelet aggregation, blood pressure, blood viscosity, and fibrinogen; replacing arachidonic acid (AA) in the cellular phospholipid pool; and inhibiting the syntheses of eicosanoids, such as prostaglandin E_2 (PGE_2) and thromboxane (TXA_2) (see excellent reviews by Kinsella et al., 1990; Simopoulos, 1991). The essentiality of dietary n-3 PUFA in growth and development has also been established recently (Simopoulos, 1991). It has been suggested that modern agribusiness and the food industry have brought abrupt changes to our dietary patterns, particularly the ratio of n-6 to n-3 PUFA in the diet (Simopoulos, 1991). The overwhelmingly high consumption of n-6 PUFA and low intake of n-3 PUFA might result in an imbalance of cellular and subcellular fatty acid compositions which favor atherogenesis.

Another type of unsaturated fatty acids, the monounsaturates, have recently been recognized for their cholesterol lowering effects. Early studies indicated that monounsaturated fatty acids were neutral in affecting serum cholesterol (Keys et al., 1965; Hegsted et al., 1965). However, more recent studies indicated that monounsaturates, mainly oleic acid, were as effective as LA in lowering serum TC as well as LDL-C

(Mattson and Grundy, 1985; Mensink and Katan, 1989). Furthermore, unlike LA, oleic acid did not decrease HDL-C (Mattson and Grundy, 1985).

Dietary fats also interact with dietary cholesterol in influencing plasma and lipoprotein cholesterol levels. Schonfeld et al. (1982) reported changes in serum TC and LDL-C of those who were fed diets varying in cholesterol levels (300, 750, 1500 mg/d) and in polyunsaturated to saturated fatty acid ratios (P/S = 0.4, 0.8, 2.5). With P/S = 0.4, diets containing both 750 and 1500 mg cholesterol/d elevated LDL-C significantly. When P/S ratio was elevated to 0.8, diets with 1500 mg cholesterol/d increased LDL-C, but not diets with 750 mg cholesterol. At P/S ratio of 2.5, dietary cholesterol as high as 1500 mg cholesterol/d had little effect on LDL-C levels.

1.4.2. Dietary Carbohydrates

The absorbable carbohydrates include mono-, di-, and polysaccharides. Early works designated carbohydrates neutral in their effects on serum cholesterol (Keys et al., 1965; Hegsted et al., 1965). In other words, when saturated fat is replaced by carbohydrates, a fall in LDL-C level can be expected. This notion was confirmed by recent studies (Grundy et al., 1988). Diets high in carbohydrates, however, paradoxically raise serum TG levels and reduce HDL-C (Ginsberg et al., 1976; Grundy et al., 1988).

The non-digestable carbohydrates can be divided into insoluble and soluble fibres. The insoluble fibres such as cellulose, hemicellulose, and lignin do not affect plasma cholesterol levels. The soluble fibres such as gums, pectins, and mucilages, have hypocholesterolemic effects

(Behall et al., 1984). Good sources for those soluble fibres include citrus fruits, barley, oats, dry beans, and peas. Dietary soluble fibres may interfere with the re-absorption of bile acids and cholesterol through the enterohepatic cycle (Stone, 1990).

1.4.3. Dietary Proteins

The type of protein affects the levels of plasma and lipoprotein cholesterol levels in both humans and experimental animals (Carroll and Hamilton, 1975; Meinertz et al., 1989; Terpstra et al., 1991). Animal proteins such as casein tend to elevate plasma total and VLDL and LDL cholesterol levels whereas substitution of animal proteins by plant proteins such as soybean protein lowers plasma cholesterol. In most experimental animals and humans, however, the effects of dietary protein are apparent only in the presence of dietary cholesterol (Meinertz et al., 1989; Terpstra et al., 1991). For instance, in normal lipidemic subjects, diets containing either soy protein or casein had no effect on plasma cholesterol levels when daily cholesterol intake was low (less than 100 mg/day) (Meinertz et al., 1989). On the other hand, when dietary cholesterol was increased to 500 mg/day, plasma total and LDL cholesterol levels were significantly higher and HDL-C lower in subjects on casein diets than the values of those on soy protein diets. Given the high daily cholesterol intake in the Western diet (450 mg per person), it has been suggested that the type of dietary protein may be an important dietary variable influencing plasma and lipoprotein cholesterol levels and atherosclerotic risks.

1.4.4. Alcohol

Considerable observational data link moderate alcohol consumption with decreased mortality from CHD (Henekens et al., 1979; LaPorete et al., 1980). This has been attributed, in part, to a positive association of HDL-C levels with alcohol consumption (Castelli et al., 1977; Hulley and Gordon, 1981). Recent studies have demonstrated that alcohol consumption raises apo A-I and A-II, the two major apolipoproteins of nascent HDL particles (Taskinen et al., 1987; Moore et al., 1988).

1.5. Objectives of This Thesis

Chicken eggs have been used extensively as the source of dietary cholesterol in most animal and clinical studies examining the effects of dietary cholesterol. In those studies, mega doses of eggs or egg yolks are generally used. For example, four to six eggs a day are consumed in several clinical studies. One large egg or egg yolk contains about 6 grams of lipids (30% of which are phospholipids) in addition to about 220 mg of cholesterol. Four to six eggs will provide 24 to 36 grams of lipids to daily consumption. The possible effects of egg or yolk components other than cholesterol such as yolk lipids on cholesterol metabolism, however, are often neglected. On the other hand, egg consumption has consistently led to elevated HDL-C levels. Although repeatedly mentioned in various reviews, no attempts have been made to understand which egg components cause the elevation of HDL-C, leaving an unanswered paradox that egg consumption could actually protect against atherosclerosis. Therefore, the first part of the thesis will examine how egg yolk components such as yolk neutral lipids and phospholipids affect the plasma and lipoprotein cholesterol levels. Rats were chosen as the experimental model due to their long-established suitability for cholesterol studies.

The second part of this thesis explores the possibility of altering the cholesterolemic properties of chicken eggs by biologically modifying the composition of certain yolk components. In light that dietary n-3 PUFA play specific roles in the amelioration and prevention of atherosclerosis, studies are carried out to enrich the egg yolk with n-3 PUFA. The influences of this modification on the cholesterolemic actions of the yolk, the internal and sensory qualities of the egg, and the feasibility of practical application is investigated.

Table 1.1. Concentration and percentage distribution of cholesterol in humans[1]

System	Concentration (% wet weight)	Percentage of total body cholesterol
Brain and nervous	2.0	22
Connective tissue (including adipose) and body fluid	.25	22
Muscle	.1	21
Skin	.3 - .7	11
Blood	.2	8
Bone marrow	.25	5
Liver	.3	4
Alimentary tract	.15	3
Lungs	.2	1
Kidneys	.3	1
Adrenal glands	2.6 - 15	1

[1] adapted from Sabine, 1977.

Table 1.2. Characteristics of lipoproteins of normal human plasma[1]

Lipoprotein class	Density (g/mL)	Electrophoretic mobility	Particle diameter (A)
Chylomicrons	< .95	Origin	10^3 - 10^4
VLDL	.95 - 1.006	pre-ß	250 - 750
IDL	1.006 - 1.019	pre-$ß_1$	250
LDL	1.019 - 1.063	ß	200 - 250
HDL_2	1.063 - 1.120	α_1	70 - 120
HDL_3	1.120 - 1.210	α_1	50 - 100

[1] Adapted from Gibbons et al., 1982. Abbreviations: VLDL, very-low-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins.

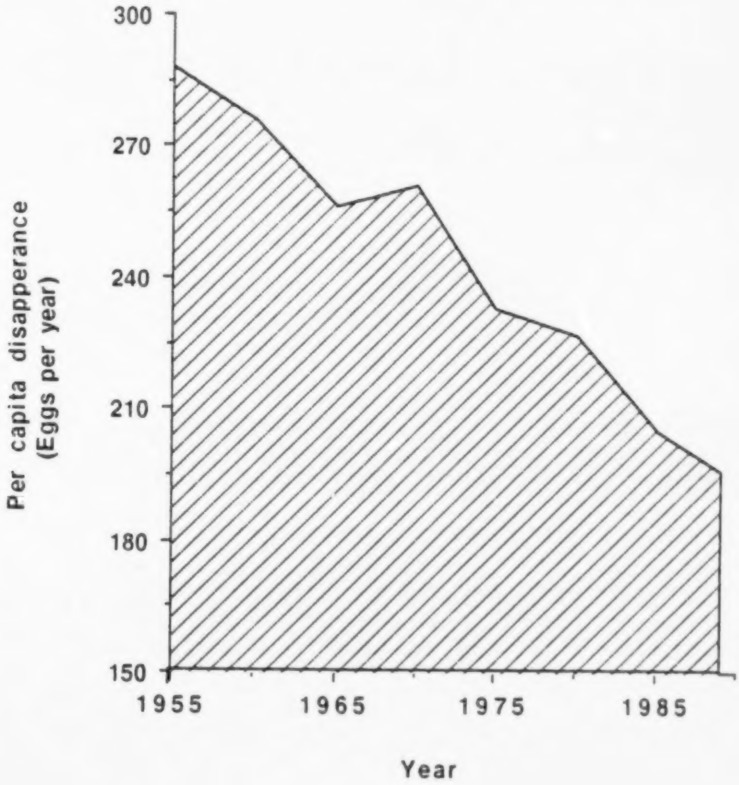
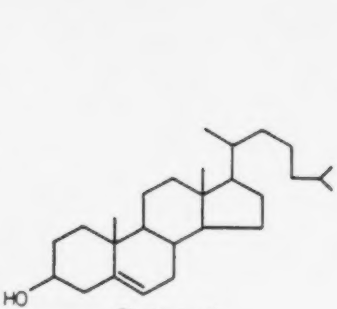
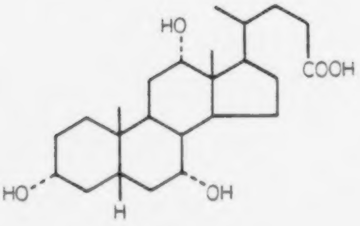


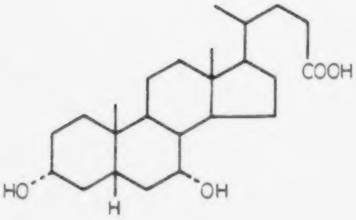
Figure 1.1 The per capita egg consumption in Canada during the past four decades (Statistics Canada, 1990).



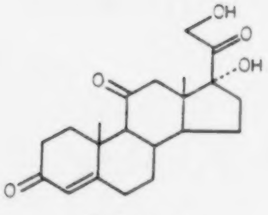
Cholesterol


HO
COOH
HO
OH
H

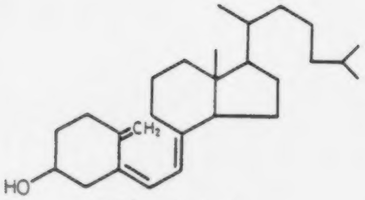
Cholic acid



Chenodeoxycholic acid



Cortisone



Cholecalciferol
(9, 10-Seco-5,7,10 (19)-
cholestatrien-3β- ol)

Figure 1.2 The structure of cholesterol and related componds

1.6. References

Ahrens, E H., J. Hirsch, W. Insull, T. T. Tsaltas, R. Blomstrand, and M. L. Peterson, 1957. The influence of dietary fats on serum-lipid levels in man. Lancet 1:943-953.

Anitschkow, N., and S. Chalatow, 1913. Veber experimentelle cholesterinseatose und ihre vedeutung fur die entstehung einiger pathologisher prozesse. Zentralbl. Allg. Pathol. Pathol. Anat. 24:1-9.

Anon, 1976. Migrants and cardiovascular disease. British Med. J. 2:1423-1424.

Applebaum-Bowden, D., S. M. Haffner, E. Hartsook E, K. H. Luk, J. J. Albers, and W. R. Hazzard, 1984. Down-regulation of the low-density lipoprotein receptor by dietary cholesterol. Am. J. Clin. Nutr. 39:360-367.

Bang, H. O., and J. Dyerberg, 1972. Plasma lipids and lipoproteins in Greenlandic West-coast Eskimos. Acta. Med. Scand. 192:85-94.

Bang, H. O., J. Dyerberg, and N. Hjorne, 1976. The composition of food consumed by Greenland Eskimos. Acta. Med. Scand. 200:69-73.

Becker, N., D. T. Illingworth, P. Alaupovic, W. E. Connor, and E. E. Sundberg, 1983. Effects of saturated, monounsaturated, and omega-6 polyunsaturated fatty acids on plasma lipids, lipoproteins, and apoproteins in human. Am. J. Clin. Nutr. 37:355-360.

Behall, K. M., K. H. Lee, and P. B. Moser, 1984. Blood lipids and lipoproteins in adult men fed four refined fibers. Am. J. Clin. Nutr. 39:209.

Benditt, E. P., and J. M. Benditt, 1973. Evidence for a monoclonal origin of human atherosclerotic plagues. Proc. Natl. Acad. Sci. USA. 70:1753-1756.

Berthelot, M., 1859. Sur plusieurs alcools nouveaux. Ann. Chim. Phys. 56:51-98.

Beveridge, J. M. R., W. F. Connel, G. A. Mayer, and H. L. Haust, 1960. The response of man to dietary cholesterol. J. Nutr. 71:61-65.

Beynen, A. C., and M. B. Katan, 1985. Reproducibility of the variations between humans in the response of serum cholesterol to cessation of egg consumption. Atherosclerosis 57:19-31.

Bloch, K., 1985. Cholesterol: Evolution of Structure and Function. Pages 1-24 in: Biochemistry of Lipid and Membranes. D. E. Vance, and J. E. Vance, ed. The Benjamin/Cummings Publishing Company, Inc. Menlo

Park, California.

Bonanome, A., and S. M. Grundy, 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N. Engl. J. Med. 318:1244-1248.

Bronte-Stewart, B., A. Antonis, L. Eales, and J. F. Brock, 1956. Effects of feeding different fats on serum-cholesterol level. Lancet 1: 521-527.

Brown, M. S., and J. L. Goldstein, 1983. Receptor-mediated endocytosis: Insights from the lipoprotein receptor system. Proc. Natl. Acad. Sci. USA. 76:3330-3337.

Brown, M. S., S. K. Basu, J. R. Falck, y. K. Ho, and J. L. Goldstein, 1980. The scavenger cell pathway for lipoprotein degradation: Specificity of the binding site that mediates the uptake of negatively charged LDL by macrophages. J. Supramolec. Str. 13:67-81.

Caplan, B. A., and C. J. Schwartz, 1973. Increased endothelial cell turnover in areas of in vivo Evans Blue uptake in the pig aorta. Atherosclerosis. 17:401-417.

Carew, T. E., R. P. Saik, K. H. Johansen, C. A. Dennis, and D. Steinberg, 1976. Low density and high density lipoprotein turn over following portacaval shunt in swine. J. Lipid Res. 17: 441-450.

Carew, T. E., J. T. Doyle, and T. Gordon, 1977. Alcohol and blood lipids. The Cooperative Lipoprotein Phenotyping Study. Lancet 2:62-64.

Carroll, K. K., and R. M. G. Hamilton, 1975. Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. J. Food Sci. 40:18-23.

Castelli, W. P., J. T. Doyle, and T. Gordon, 1977. Alcohol and blood lipids. The Cooperative Lipoprotein Phenotyping Study. Lancet 2:153-155.

Castelli, W. P., P. W. F. Wilson, D. Levy, and K. Anderson, 1990. Serum lipids and risk of coronary artery disease. Atherosclerosis Rev. 21:7-19.

Chevreul, M., 1816. Sixieme memoire: Examen des graisses d'homme, demouton, de boeuf, de jaguar et d'oie. Paper read at the Academie des Sciences, Paris. 339-372.

Clarkson, T. B., M. G. Bond, K. J. McLaughlin, and J. K. Sawyer, 1984. A study of atherosclerosis regression in Macaca mulatta. V. Changes in abdominal aorta and carotid and coronary arteries from animals with atherosclerosis induced for 38 months and then regressed for 24 or 48 months at plasma cholesterol concentrations of 300 or 200 mg/dl. Exp. Mol. Pathol. 41:96-118.

Clarkson, T. B., N. D. M. Lehner, W. D. Wagner, R. W. St. Clair, M. G. Bond, and B. C. Bullock, 1979. A study of atherosclerosis regression in Macaca mulatta. I. Design of experiment and lesion induction. Exp. Mol. Pathol. 30:360-385.

Connor, W. E., 1970. The effects of dietary lipid and sterols on the sterol balance. Pages 253-261 in Atherosclerosis: Proceedings of the Second International Symposium, R. J. Jones, ed. Springer-Verlag, New York, NY.

Connor, W. E., 1982. Letters to the editor. Am. J. Clin. Nutr. 36:1261-1264.

Connor, W. E., D. B. Stone, and R. E. Hodges, 1964. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. J. Clin. Invest. 43:1691-1696.

Connor, W. E., and D. S. Lin., 1974. The intestinal adsorption of dietary cholesterol by hypercholesterolemic (type II) and normocholesterolemic humans. J. Clin. Invest. 53: 1062-1070.

Cortese, C., Y. Levy, E. D. Janus, P. R. Turner, S. N. Rao, N. E. Miller, and B. Lewis, 1983. Modes of action of lipid-lowering diets in man: studies of apolipoprotein B kinetics in relation to fat consumption and dietary fatty acid consumption. Atherosclerosis. 20:347-364.

Cryer, A., 1983. Lipoprotein lipase-endothelial interactions. Pages 245-74 in: Biochemical Interactions at the Endothelium. A. Cryer, ed. Elsevier, Amsterdam.

Cushing, S. D., J. A. Berliner, A. J. Valente, M. C. Territo, N. Mahamad, F. Parhami, R. Garrity, C. G. Schwartz, and A. M. Fogelman, 1990. Minimally modified low density lipoprotein induces monocyte chemotactic protein I in human endothelial cells and smooth muscle cells. Proc. Natl. Acad. Sci. USA. 87:5134.

Danielsson, H., and K. Einarsson, 1969. Formation and metabolism of bile acids. Pages 279-315 in: The Biological Basis of Medicine. E. E. Bittar and N. Bittar, ed. Academic press, London.

Davison, A. N., 1970. Vol.18, Pages 293-329 in: Comprehensive Biochemistry. M. Florkin, and E. H. Stotz, ed. Elsevier, Amsterdam.

Dawber, T. R., 1980. The Framingham Study. The Epidemiology of Atherosclerotic Disease. Cambridge, Mass., Harvard Univ. Press.

Dawber, T. R., R. J. Nickerson, F. N. Brand, and J. Pool, 1982. Eggs serum cholesterol and coronary heart disease. Am. J. Clin. Nutr. 36:617-625.

de Fourcroy, M., 1789. Examen chimique de la substance feuilletee et cristalline contenue dans les calculs biliaires, et de la nature des concretions cystiques cristallisees. Ann. Chim. 242-252.

Dietschy, J. M., 1978. General principles governing movement of lipids across biological membranes. Pages 1-28 in: Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy A. M. Gotto, and J. A, Ontko, ed. Bethesda, Maryland, American Physiological Society.

Edington, J., M, Geekie, R. Carter, L. Benfield, K. Fisher, M. Ball, and J. Mann, 1987. Effect of dietary cholesterol concentration in subjects following reduced fat high fiber diet. Brit. Med. J. 294:333-336.

Edwards, P. A., H. Muroya, and R. G. Gould, 1972. In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. J. Lipid Res. 13:396-401.

Egusa, G., W. F. Beltz, S. M. Grundy, and B. V. Howard, 1985. The influence of obesity on the metabolism of apolipoprotein B in man. J. Clin. Invest. 76:596-603.

Elovson, J., 1965. Immediate fate of albumin bound [1-^{14}C] stearic acid following its intraportal injection into carbohydrate refed rats. Early course of desaturation and esterification. Biochim. Biophys. Acta. 106:480-494.

Field, F. J., N. T. P. Kam, and S. N. Mathur, 1990. Regulation of cholesterol metabolism in the intestine. Gastroenterol. 99:539-551.

Fielding, C. G., and P. E, Fielding, 1982. Cholesterol transport between cells and body fluids: Role of plasma lipoproteins and the plasma cholesterol esterification system. Med. Clin. North Am. 66:363-373.

Fielding, C. J., and P. E. Fielding, 1985. Metabolism of cholesterol and lipoproteins. Pages 404-474 in: Biochemistry of Lipids and Membranes. D. E. Vance and J. E. Vance, ed. The Benjamin/Cummings Publishing Company, Inc. Menlo Park, California.

Fogelman, A. M., I. Shechter, J. Seager, M. Hokom, J. S. Child, and P. A. Edwards, 1980. Malondialdehyde alteration of low density lipoprotein leads to cholesteryl ester accumulation in human monocyte-macrophages. Proc. Natl. Acad. Sci. USA. 77:2214-2218.

Fox, J. C., H. C. McGill, Jr., K. D. Carey, and G. S. Getz, 1987. In vivo regulation of hepatic LDL receptor mRNA in the baboon: differential effects of saturated and unsaturated fat. J. Biol. Chem. 262:7014-7020.

Fry, D. L., 1973. Responses of the arterial wall to certain physical factors. Page 93 in Atherogenesis: Initiating factors. Ciba Foundation Symposium 12, Elsevier, Amsterdam, London, New York.

Garcia-Palmieri, M. R., P. Sorlie, J. Tillotson, R. Costas, Jr., E. Cordero, and M. Rodriguz, 1980. Relationship of dietary intake to subsequent coronary heart disease incidence: the Puerto Rico Heart Program. Am. J. Clin. Nutr. 33:1818-1827.

Gibbons, G. F., K. A. Mitropoulos, and N. B. Myant, 1982. Biochemistry of cholesterol. Pages 109-130. Elsevier Biomedical Press, New York, NY.

Ginsberg, H., J. M. Olefsky, G. Kimmerling, P. Crapo, and G M. Reaven, 1976. Induction of hypetriglyceridemia by a low-fat diet. J. Clin. Endocrinol. Metab. 42:729-735.

Glueck, C. J., G. Waldman, and D. K. McClish, et al. 1982. Relationship of nutrient intake to lipids and lipoproteins in 1234 white children. The lipid Clinics Prevalence Study. Arteriosclerosis 2:523-536.

Goldstein, J. L., and M. S. Brown, 1982. The LDL receptor defect in familial hypercholesterolemia: implications for pathogenesis and therapy. Med. Clin. North Am. 66:335-362.

Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown, 1979. Binding site in macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc. Natl. Acad. Sci. USA. 76:333-337.

Gordon, D. J., K. M. Salz, K. J. Roggenkamp, and F. A. Franklin, Jr. 1982a. Dietary determinants of plasma cholesterol change in the recruitment phase of the Lipid Research Clinics Coronary Primary Prevention Trial. Arteriosclerosis 2:537-548.

Gordon, T., A. Kagan, and M. Garcia-Palmieri, et al., 1981. Diet and its relation to coronary heart disease and death in three populations. Circulation 63:500-515.

Gordon, T., M. Fisher, N. Ernst, and B. M. Rifkind, 1982b. Relation of diet to LDL cholesterol, VLDL cholesterol, and plasma total cholesterol and triglycerides in white adults. The Lipid Research Clinics Prevalance study. Arteriosclerosis, 2:502-512.

Goto, Y., and Moriguchi, E. H., 1990. Diet and ischemic heart disease in Japan. Atherosclerosis Rev. 21:33.

Grundy, S. M., 1986. Liver, lipoprotein, and atherosclerosis: An overview. Pages 1-12 in : Bile Acids and Atherosclerosis. S. M. Grundy, ed. Raven Press. New York, NY.

Grundy, S. M., and A. L. Metzger, 1972. A physiological method for estimation of hepatic secretion of biliary lipids in man. Gastroenterol. 62:1200-1217.

Grundy, S. M., and G. L. Vega. 1988. Plasma cholesterol responsiveness to saturated fatty acids. Am. J. Clin. Nutr. 47:822-824.

Grundy, S. M., and H. Y. I. Mok, 1976. Effect of low dose phytosterols on cholesterol absorption in man. Pages 112-118 in: Lipoprotein Metabolism. H. Geten, ed. Berlin, Springer-Verlag.

Grundy, S. M., and H. Y. I. Mok, 1977. Determination of cholesterol absorption in man by intestinal perfusion. J. Lipid Res. 18: 263-271.

Grundy, S. M., R. N. Lea Florentin, R. D. Dana Nix, and R. N. Marjorie F Whelan, 1988. Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. Am. J. Clin. Nutr. 47:965-699.

Grundy, S. M., and M. A. Denke, 1990. Dietary influences on serum lipids and lipoproteins. J. Lipid Res. 31:1149-1172.

Haberland, M. E., C. L. Olch, and A. M. Fogelman, 1984. Role of lysines in mediating interaction of modified low density lipoproteins with the scavenger receptor of human monocyte macrophages. J. Biol. Chem. 259:11305-11311.

Hardison, W. G. M., and J. T. Apter, 1972. Micellar theory of biliary cholesterol excretion. Am. J. Phys. 222:61-67.

Hashim, S. A., A. A. Arteaga, and T. B. van Itallie, 1960. Effect of a saturated medium-chain triglyceride on serum-lipids in man. Lancet. 1:1105-1108

Health and Welfare Canada, 1990. Nutrition Recommendations. Pages 53-70. Canada Government Publishing Centre Supply and Services Canada, Ottawa, Canada.

Hegsted, D. M., 1986. Serum-cholesterol response to dietary cholesterol: a re-evaluation. Am. J. Clin. Nutr. 44:299-305.

Hegsted, D. M., R. B. McGandy, M. L. Myer, and F. J. Stare, 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17:281-295.

Hennekens, C. H., W. Willett, and B. Rosner, et al. 1979. Effect of beer, wine, and liquor in coronary deaths. JAMA. 242:1973-1974.

Henricksen, T., E. M. Mahoney, and D. Steinberg, 1981. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: Recognition by receptors for acetylated low density lipoproteins. Proc. Natl. Acad. Sci. USA. 78:6499.

Hollander, W., M. Colomb, and C. Small, 1990. Further evidence that atherosclerosis is an autoimmune disease. Arteriosclerosis. 10:756a.

Hulley, S. B., and S. Gordon, 1981. Alcohol and high-density lipoprotein cholesterol. Causal inference from diverse study design. Circulation 64(suppl III):III-57-63.

Hunter, C. F., and V. W. Rodwell., 1980. Regulation of vertebrate liver HMG-CoA reductase via reversible modulation of its catalytic activity. J. Lipid Res. 21:399-405.

Innerarity, T. L., R. E. Pitas, and R. W. Mahley, 1980. Receptor binding of cholesterol-induced high-density lipoproteins containing predominantly apoprotein E to cultured fibroblasts with mutations at the low density receptor locus. Biochemistry. 19:4359-4365.

Jackson, R. L., M. L. Kashyap, R. L. Barnhart, C. Allen, E. Hogg, and C. J. Glueck, 1984. Influence of polyunsaturated and saturated fat on plasma lipids and lipoproteins in man. Am. J. Med. 66:105-109.

Kato, H., J. Tillotson, M. Z. Nichaman, G. G. Rhoades, and H. B. Hamilton, 1973. Epidemiological studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: Serum lipids and diet. Am. J. Epidemiol. 97:372-385.

Kesaniemi, Y. A., W. F. Beltz, and S. M. Grundy, 1985. Comparisons of metabolism of apoliproprotein B in normal subjects, obese patients and patients with coronary heart disease. J. Clin. Invest. 76:586-595.

Keys, A., 1984. Serum-cholesterol response to dietary cholesterol. Am. J. Clin. Nutr. 40:351-359.

Keys, A., J. T. Anderson, and F. Grande, 1965. Serum cholesterol responses to changes in the diet. II. The effect of cholesterol in the diet. Metabolism 14:776-787.

Kinsell, L. W., G. C. Micheals, J. W. Partridge, L. A. Boling, H. E. Balch, and G. C. Cochrane, 1953. Effect upon serum cholesterol and phospholipids of diets containing large amount of vegetable fat. J. Clin. Nutr. 1:231-244.

Kinsella, J. E., B. Lokesh, and R. A. Stone, 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52:1-28.

Knuiman, J. T., S. Westenbrink, and L. Van der Heyden, et al. 1983. Determinants of total and high-density lipoprotein cholesterol in boys from Finland, the Netherlands, Italy, the Philippines and Ghana with special reference to diet. Human Nutr: Clin. Nutr. 376:237-254.

Kovanen, P. T., M. S. Brown, S. K. Basu, D. W. Bilheimer, and J. L. Goldstein, 1981. Saturation and suppression of hepatic lipoprotein receptors: a mechanism for the hypercholesterolemia of cholesterol-fed rabbits. Proc. Natl. Acad. Sci. USA 78:1396-1400.

Kroomhout, D., E. B. Boschieter, and C. de Lezenne Coulander, 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312:1205-1209.

Kudchodkar, B. J., 1973. Absorption of dietary cholesterol in man. Metabol. 22:155-163.

Kuschi, L. H., R. A. Lew, and F. J. State, et al. 1985. Diet and 20-year mortality from coronary heart disease. The Ireland-Boston Diet-Heart Study. N. Engl. J. Med. 312:811-888.

LaPorete, R.E., J. K. Cresarta, and L. H. Kuller, 1980. The relationship of alcohol consumption to atherosclerotic heart disease. Prev. Med. 9:22-40.

LaRosa, J. C., D. Hunninghake, and D. Bush, et al., 1990. The cholesterol facts: a summary of the evidence relating Dietary fats, serum cholesterol, and coronary heart disease. Circulation 81: 1721-1733.

Lecanu, M. L. R., 1838. Etudes chimiques sur le sang humain. Ann. Chim. Phys. 67:54-70.

Lin, D. S., and W. E. Connor, 1980. The long-term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption and the sterol balance in man: The demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. J. Lipid Res. 21: 1042-1052.

Loscalzo, J., J. Fredman, R. M Rudd, I. Barsky-Vasserman, and D. E. Vaughan, 1987. Unsaturated fatty acids enhance low density lipoprotein uptake and degradation by peripheral blood mononuclear cells. Arteriosclerosis 7:450-455.

Mahley, R. W., and T. L. Innerarity, 1983. Lipoprotein receptors and cholesterol homeostasis. Biochim. Biophys. Acta. 737:197-222.

Mattson, F. H., and S. M. Grundy, 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. lipid Res. 26:194-202.

McGill, H. C. Jr., 1979. The relationship of dietary cholesterol to serum cholesterol concentration and to atherosclerosis in man. Am. J. Clin. Nutr. 32:2664-2702.

McGill, H. C., J. C. Geer, and R. L. Holman, 1957. Sites of vascular vulnerability in dogs demonstrated by Evans Blue. AMA Arch. Pathol. 64:303.

McGill, H. C., Jr., C. A. McMahan, A. W. Kruski, J. L. Kelley, and G. E. Mott, 1981. Responses of serum lipoproteins to dietary cholesterol and type of fat in the baboon. Arteriosclerosis. 5:337-344.

Meinertz, H., K. Nilausen and O. Faergeman, 1989. Soy protein and casein in cholesterol-enriched diets: effects on plasma lipoproteins in normolipidemic subjects. Am. J. Clin. Nutr. 50:786-793.

Mensink, R. P., and M. B. Katan, 1989. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high density lipoprotein cholesterol in healthy men and women. N. Engl. J. Med. 321:436-441.

Miller, N. E., D. B. Weinstein, and D. Steinberg, 1977. Binding, internalization, and degradation of high density lipoprotein by cultured normal human fibroblasts. J. Lipid Invest. 47:166-174.

Moore, J. H., 1987. Biochemical aspects of the relationships between dietary cholesterol, blood cholesterol and ischaemic heart disease. Pages 27-56 in: Egg Quality-Current Problems and Recent Advances. R. G. Wells, and C. G. Belyavin, ed. Butterworth & Co. Ltd, London, England.

Moore, R. D., C. R. Smith, P. O. Kwiterovich, and T. A. Pearson, 1988. Effect of low-dose alcohol use versus abstention on apolipoproteins A-1 and B. Am. J. Med. 84:884-890.

Moreng, R. E., and J. S. Avens, 1985. Pages 15-46 in: Poultry Science and Production. Reston Publishing Company, Inc., Reston, VA.

Myant, N. B., and K. A. Mitropoulos, 1977. Cholesterol 7α-hydroxylase. J. Lipid Res. 18:135-153.

Nelson, A. M., 1972. Diet therapy in coronary disease. Effect on mortality of high-seafood, fat-controlled diet. Geriatrics 12:103-116.

Nestel, P., N. Tada, T. Billington, M. Huff, and N. Fidge, 1982. Changes in very low density lipoproteins with cholesterol loading in man. Metabolism 31:398-405.

Nestel, P. J., M. Reardon, and T. Billington, 1979. In vivo transfer of cholesterol esters from high density lipoproteins to very low density lipoproteins in man. Biochim. Biophys. Acta. 573:403-407.

Oh, S. Y., and L. T. Miller, 1985. Effect of dietary egg on variability of plasma cholesterol levels and lipoprotein cholesterol. Am. J. Clin. Nutr. 42:421-431.

Oram, J. F., E. A. Brinton, and E. L. Bierman, 1983. Regulation of high density lipoprotein receptor activity in cultured human skin fibroblasts and human arterial smooth muscle cells. J. Clin. Invest. 72:1611-1621.

Oster, K. A., 1982. Letters to the editor. Am. J. Clin. Nutr. 36:1259-1261.

Parthasarathy, S., 1987. Oxidation of low density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. Biochim. Biophys. Acta. 917:337-340.

Paul, R., C. S. Ramesha, and J. Ganguly, 1980. On the mechanism of hypocholesterolemic effects of polyunsaturated lipids. Adv. Lipid Res. 17:155-171.

Quinn, M. T., S. Parthasarathy, L. G. Fong, and D. Steinberg, 1987. Oxidatively modified low density lipoprotein: a potential role in recruitment and retention of monocyte/macrophages in atherogenesis. Proc. Natl. Acad. Sci. USA. 84:2995-2998.

Reinitzer, F., 1888. Beitrage zur Kenntniss des cholesterins. Sitzber. Ost. Akad. Wiss. 97:167-187.

Rodwell, V. W., J. L. Nordstrom, and J. J. Mitschelen, 1976. Regulation of HMG-CoA reductase. Adv. Lipid Res. 14:1-74.

Rudel, L.L., J. S. Parkes, and M. G. Bond, 1985. LDL heterogeneity and atherosclerosis in nonhuman primates. Ann. NY Acad. Sci.454:248-253.

Sabine, J. R., 1977. Cholesterol. Pages 5-28. Marcel Dekker, Inc. New York, NY.

Sacks, F. M., J, Salazar, L. Miller, J. M. Foster, M. Sutherland, K. W. Samonds, J. J. Albers, and E. H. Kass, 1984. Ingestion of egg raises plasma low density lipoproteins in free-living subjects. Lancet i, 647-649.

Schonfeld, G., W. Patsch, L. L. Ridel, C. Nelson, M. Epstein, and R. E. Olson, 1982. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. J. Clin. Invest. 69:1072-1080.

Schwartz, C. J., A. J. Valente, E. A. Sprague, J. L. Kelley, and R. M. Nerem, 1991. The pathogenesis of atherosclerosis: an overview. Clin. Cardiol. 14:I-1-16.

Schwartz, C. G., E. A. Sprague, A. J. Valente, J. L. Kelley, E. H. Edwards, and C. A. Suenram, 1990. Inflammatory components of the human atherosclerotic plaque. Pages 107 in: Pathobiology of the Human Atherosclerotic Plaque. S. Glagov, W. P. Newman, III, and S. A. Schaffer, ed. Springer-Verlag. New York, NY.

Schwartz, C. J., J. L. Kelley, R. M. Nerem, E. A. Sprague, M. M. Rozek, A. J. Valente, E. H. Edwards, A. R. S. Prasad, J. J. Kerbacher, and S. A. Logan, 1989. Pathophysiology of the atherogenic process. Am. J. Cardiol. 64:23G-30G.

Schwartz, C. J., and J. R. A. Mitchell, 1962. Cellular infiltration of the human arterial adventitia associated with atheromatous plaques.

Circulation. 26:73-78.

Sexton, R. C., S. R. Panini, F. Azran, and H. Rudney, 1983. Effects of 3ß-[2-(diethylamino)ethoxy]androst-5-en-17-one on the synthesis of cholesterol and ubiquinone in rat epithelial cell cultures. Biochemistry 22:5687-5692.

Shekelle, R. B., S. A. MacMillan, and O. Paul, et al., 1981. Diet, serum cholesterol and death from coronary heat disease. The Western Electric Study. N. Engl. J. Med. 304:65-70.

Shepherd, J., C. J. Packard, J. R. Patsch, A. M. Gotto, Jr., and O. D. Taunton, 1978. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoprotein and the metabolism of apolipoprotein A-I. J. Clin. Invest. 60:1582-1592.

Shrimpton, D. H., 1987. The nutritive value of eggs and their dietary significance. Pages 11-25 in: Egg Quality - Current Problems and Recent Advances. R. G. Wells, and C. G. Belyavin, ed. Butterworth & Co. Ltd., London, England.

Shroepfer, G. J., 1981. Sterol biosynthesis. Ann. Rev. Biochem. 50:585-621.

Simopoulos, A. P., 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Nutr. 54:438-463.

Sinclair, H., 1956. Deficiency of essential fatty acids and atherosclerosis, etcetera. Lancet 1: 381-383.

Soutar, A. K., and N. B. Myant, 1979. Pages 55-119 in: Chemistry of Macromolecules IIB. Macromolecular Complexes. International Review of Biochemistry. R. E. Offord, ed. University Park Press, Baltimore.

Spady, D K., and J. M. Dietschy, 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster and rat. J. Lipid. Res. 24:303-315.

Stalenhoef, A. F., M. J. Malloy, J. P. Kane, and R. J. Havel, 1984. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. Proc. Natl. Acad. Sci. USA. 81:1839-1843.

Stamler, J., and Shekelle, R., 1988. Dietary cholesterol and human coronary heart disease. Arch. Pathol. Lab. Med. 112:1032-1040.

Stehbens, W. E., 1989. The controversial role of dietary cholesterol and hypercholesterolemia in coronary heart disease and atherogenesis. Pathology 21:213-221.

Steinberg, D., 1989. The cholesterol controversy is over. Why it take so long? Circulation 80:1070-1078.

Steinbrecher, U. P., S. Parthasarathy, D. S. Leake, J. L. Witztum, and D. Steinberg, 1984. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipid. Proc. Natl. Acad. Sci. USA. 81:3883-3887.

Stone, N. J., 1990. Diet, lipid, and coronary heart disease. Endocrinol. Metab. Clin. North Am. 19:321-344.

Strong, J. P., 1976. Atherosclerosis in primates. Introduction and overview. Primates Med. 9:1-15.

Stryer, L., 1981. Pages 457-484 in: Biochemistry. W. H. Freeman and Company, San Francisco, CA.

Tall, D. M., and D. M. Small, 1980. Body cholesterol removal: Role of plasma high-density lipoproteins. Adv. Lipid Res. 17:1-51.

Tanaka, R. D., P. A. Edwards, S. F. Lan, and A. M. Fogelman, 1983. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in avian myeoblasts. Mode of action of 25-hydroxycholesterol. J. Biol. Chem. 258:13331-13339.

Taskinen, M. R., E. A. Nikkila, M. Valimaki, et al., 1987. Alcohol-induced changes in serum lipoproteins and their metabolism. AM. Heart J. 113:458-469.

Terpstra, A. H. M., J. C. Holmes, and R. J. Nicolosi, 1991. The hypocholesterolemic effect of dietary soybean protein vs casein in hamsters fed cholesterol-free or cholesterol-enriched semipurified diets. J. Nutr. 121:944-947.

Texon, M., 1989. The cholesterol-heart disease hypothesis (critique)-time to change course? Bull. N. Y. Acad. Med. 65:836-841.

The Framingham Study-An epidemiological investigation of cardiovascular disease. 1970. Section 24. The Framingham Diet Study. Washington, DC: US Dept. of Health, Education, and Welfare.

Treadwell, C. R., and G. V. Vahouny, 1968. Cholesterol absorption. Pages 1407-1438 in: Handbook of Physiology, Section 6, Vol. 3, C. F. Code, ed. Washington, Am. Physiol. Soc.

Uemura, K., and Z. Pisa, 1985. Recent trends in cardiovascular disease mortality in 27 industrialized countries. World Health Statistics Quarterly, 38:142-162.

Vega, G. L., E. Groszek, R. Wolf, and S. M. Grundy, 1982. Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. J. Lipid Res. 23:811-822.

Vorster, H. H., N. Silvis, C. S. Venter, et al., 1987. Serum cholesterol, lipoproteins and plasma coagulation factors in South African blacks on a high-egg but low-fat intake. Am. J. Clin. Nutr. 46:52-57.

Wagner, W. D., R. W. St. Clair, T. B. Clarkson, and J. R. Connor, 1980. A study of atherosclerosis regression in macaca mulatta. III. Chemical changes in arteries from animal with atherosclerosis induced for 19 months and regressed for 48 months at plasma cholesterol concentrations of 300 or 200 mg/dl. Am. J. Pathol. 100:633-650.

Wheeler, H. O., and K. K. King, 1972. Biliary excretion of lecithin and cholesterol in the dog. J. Clin. invest. 51:1337-1350.

Whyte, M., P. Nestel, and A. Macgregor, 1977. Cholesterol metabolism in Papua New Guineans. Europ. J. Clin. Invest. 7:53-60.

Wieland, H., and E. Dane, 1932. Untersuchungen uber die Konstitution der Gallensauren. 39. Mitteilung zur Kenntnis der 12-oxy-Cholansaure. Z. Physiol. Chem. 210:268-281.

Windaus, A., 1932. Uber die Konstitution des Cholesterins und der Gallensauren. Z. Physiol. Chem. 213:147-187

Windler, E., Y. S. Chao, and R. J. Havel, 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. J. Biol. Chem. 255:5475-5480.

Wissler, R. W., and D. Vesselinovitch, 1987. The development and use of animal models in atherosclerotic research. Pages 337-335 in Cardiovascular Disease. L. L. Gallo, ed. Plenum Press, New York, NY.

Worne, H. E., and L. W. Smith, 1959. Effects of certain pure long chain polyunsaturated fatty acid esters on the blood lipids in man. Am. J. Med. Sci. 237: 710-721.

Yoshimura, T., E. A. Robison, S. Tanaka, E. Appella, and E. J Leonard, 1989. Purification and amino acid analysis of two human monocyte chemoattractants produced by phytohemagglutinin stimulated human peripheral blood mononuclear leukocytes. J. Immunol. 142:1956-1962.

Zilversmith, D. B., L. B. Hughes, and J. Balmer, 1975. Stimulation of cholesterol ester exchange by lipoprotein-free rabbit plasma. Biochim. Biophys. Acta. 409:393-398.

Chapter 2. Cholesterol Content of Eggs: Methodology and Influencing Factors

Before examining the relationship between dietary egg components and ovo-cholesterol metabolism, an accurate estimation of egg cholesterol contents was required. A search of the available data on egg cholesterol content revealed a wide variation of reported values. Thus the methodology for cholesterol determination and the possible biological factors influencing egg cholesterol content were investigated.

2.1. Comparison of Four Different Methods for Egg Cholesterol Determination[1]

2.1.1. Introduction

Cholesterol content of chicken eggs has recently received far more attention than before from farmers, consumers, medical professionals, and researchers. The newest average value reported by USDA Marketing Service is 213 milligrams per large egg (mg/egg) (USDA, 1989), 22.3% lower than the previous report of 274 mg/egg (USDA, 1976). The reason for this discrepancy is not clearly defined. Cholesterol values of chicken eggs reported in the 1970s ranged from 9.21 to 22.8 milligrams per gram of yolk (mg/g yolk) and 156.6 to 387.6 mg/egg, assuming a 17-g yolk for a large egg (Cunningham et al., 1974; Nix et al., 1974; Washburn and Nix, 1974; Cotterill et al., 1977). These discrepancies and their possible causes have been overlooked.

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Several assay methods are currently available for determination of cholesterol concentrations in biological systems, especially in plasma. Few studies have compared these methods for cholesterol determination in egg yolks (Beyer and Jensen, 1989; Naber, 1989). The objective of this study was to compare four of the assay methods for cholesterol determination in chicken eggs to examine possible methodological factors contributing to variations in reported egg cholesterol values.

2.1.2. Materials and Methods

Laying hens and eggs

Ninety Single Comb White Leghorn hens, 22 wk of age, were housed in three floor pens (thirty birds per pen) and were fed a wheat and soybean meal based laying hen ration containing 16.02% protein and 2,790 kcal/kg metabolizable energy (Table 2.1). Eggs were collected on Day 180 starting from the 22nd wk. Eggs collected were pooled, and 15 eggs were randomly selected. Yolks were separated from albumen and weighed. Five yolk samples were then prepared by randomly pooling three yolks per sample, and subjected to cholesterol assay as described herein.

Cholesterol assay

About .2 g pooled yolk samples were saponified according to Abell et al. (1952) with minor modifications of incubation temperature at 50 C for 60 min (Sim and Bragg, 1977). The unsaponifiable fraction was extracted using 10 mL of hexane, and the hexane extracts were assayed for cholesterol by four methods: colorimetric method of Abell et al., (1952); enzymatic method (Jiang et al., 1990); gas chromatography (GC); and HPLC. In gas chromatography, 2 mg of 5-α-cholestane (Sigma Chemical Co., St. Louis, MO 63178) was added to each yolk sample as an internal standard (IS) before saponification.

An SE-30 capillary column (Supelco Canada Ltd., Oakville, Ontario, Canada, L6K 3V1) was used on a Varian 3400 gas chromatography (Varian Associates, Inc., Sunnyvale, CA 94089) equipped with an automatic sampling system. The operating conditions of GC were as follows: oven temperature was held at 70 C for 1 min, then programmed at 50 C/min to 300 C and held at 300 C for 5 min. Injection temperature was programmed from 80 C to 300 C at 150 C/min and held at 300 C for 7 min. Liquid CO_2 was used to cool the injector. Helium carrier gas head pressure was set at 1406.1 gram per square cm. Hydrogen and air gas flow to the detector were 30 and 300 mL/min, respectively. Detector temperature was at 300 C. A Hewlett-Packard Series 3353 laboratory automation system (Hewlett-Packard, Avondale, PA 19311) was used to integrate peak areas. Hexane extracts of yolk samples were injected directly on the GC.

For HPLC, a Supelcosil LC-18 column (Supelco Canada Ltd., Oakville, Ontario, Canada, L6K 3V1) was used with a 5 cm Pelliguard LC-18[3] guard column. The solvent was an isocratic mixture of acetonitrile and 2-propanol (3/1) and flowed at a rate of 3.0 mL/min. Samples were injected with a 25 μL loop. The ultraviolet detector was set at 210 nm. An aliquot of hexane extracts of yolk samples was dried under nitrogen and re-dissolved with ethanol before injection on the HPLC.

The thin layer chromatography with flame ionization detection (TLC-FID) method was used to analyze qualitatively the unsaponifiable extracts of egg yolks. The measurements were done with Chromarod-SIII[6] on an Iatroscan TH-10 Analyzer (Iatron Laboratories, Tokyo, Japan). Yolk unsaponifiable extracts and lipid standards of cholesterol oleate (CE), tripalmitin (TG), cholesterol (CH), and stearic acid (FA) were applied. Rods were developed in a solvent system of benzene, chloroform and formic acid (65/20/.5) for 55 min before being air dried, and scanned.

Statistical analysis

The one-way analysis of variance (ANOVA) was used to analyze the effect of cholesterol assay methods on egg cholesterol values. Assay methods were the main effects with the mean square of method by replication as the error term. Duncan's multiple range test was used to differentiate between treatment means (Steel and Torrie, 1980).

2.1.3. Results and Discussion

The cholesterol content of yolk samples determined by four different methods are presented in Table 2.2. Cholesterol values generated by the colorimetric method, expressed as either mg/g yolk or mg/egg, were significantly higher ($P<.01$) than those values determined by the enzymatic, GC, and HPLC methods.

The colorimetric technique has been the reference method for blood cholesterol assay (De La Huerga and Sherrick, 1972). This method, however, apparently overestimated the cholesterol content of chicken eggs when compared to the enzymatic, GC, and HPLC methods. When compared to the value of 274 mg/egg in the previous USDA (1976) report which is equal to 16.1 mg/g yolk assuming a 17 g yolk for a large egg, the 14.6 mg/g yolk obtained with the colorimetric method in this study is still lower. Therefore, the colorimetric method, although generating higher egg cholesterol values than the enzymatic, GC, and HPLC methods, was not the only factor that contributes to the high cholesterol values reported by the USDA (1976). On the other hand, it is obvious that the variations of reported cholesterol values in literature are caused, at least partly, by various chemical colorimetries used. A similar notion was pointed out by Naber (1989).

As the egg yolk has been subjected to alcoholic KOH saponification and hexane extraction prior to color reaction with Liebermann-Burchard reagent, it seems unlikely that the overestimation by the colorimetric method was due to the presence of triacyglycerols or free fatty acids, which are reported to interfere with the color formation in cholesterol assay (Bohac et al., 1988). This was proved by TLC-FID and GC analyses of yolk lipid extracts. As shown in Figure 2.1, no triacyglycerols or free fatty acids in the yolk extracts were detected by TLC-FID (Panel A and B). When the GC chromatogram of the yolk extracts was examined, no peak other than those of 5-α-cholestane and cholesterol was observed (Panel C), demonstrating that the yolk unsaponifiable extracts were free from free fatty acid or triacyglycerol contaminations. It was not clear from this study what caused the colorimetric method to generate values that are 23% higher than the average of those by enzymatic, GC, and HPLC methods (Table 2.2).

The enzymatic method was also originally reported for blood cholesterol determination (Allain et al., 1974) and later adopted for assay of egg and tissue cholesterol (Jiang et al., 1990). It provides excellent accuracy and precision for the determination of yolk cholesterol content when compared to GC and HPLC methods (Table 2.2). The gas chromatographic technique, with its excellent reproducibility, accuracy, and high degree of automation, should be the choice for egg cholesterol determination, especially when a large number of samples are analyzed.

2.2. Egg Cholesterol Values in Relation to the Age of Laying Hens and to Egg and Yolk Weights[1]

2.2.1. Introduction

Several laying hen and egg factors have been reported to influence the cholesterol values of chicken eggs. Turk and Barnett (1970, 1971) showed that eggs from egg strain chickens tended to have a lower yolk cholesterol concentration than those from the broiler strain. Eggs from Araucana hens averaged about 4% higher in yolk cholesterol concentration than eggs from Leghorns (Cunningham, 1977). Yolk cholesterol concentration was negatively related to rate of egg production (Bartov et al., 1971; Cunningham et al., 1974). Bair et al. (1980) reported that cholesterol values expressed in milligrams per egg (mg/egg) and milligrams per gram of yolk (mg/g yolk) decreased from the first egg to the fifth egg after the onset of production. In contrast, eggs laid by 12.5-mo old hens contained significantly more cholesterol (mg/egg) than those laid by the same hens when 11-mo-old (Spencer et al., 1978). Fertile eggs were not lower in cholesterol content than infertile eggs (Spencer et al., 1978). An inverse relationship between yolk size and cholesterol concentration was also reported (Nichols et al., 1963).

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The currently used expressions for egg cholesterol value, mg/g yolk and mg/egg, do not take into consideration the aforementioned bird and egg factors, and cause confusion in certain cases for the egg consumer. Some egg producers, for example, claim that their chicken eggs contain cholesterol as low as 125 mg/egg, which was more than 40% less than the newly reported USDA value of 213 mg/egg (USDA, 1989).

In the present study, the relationships between egg cholesterol values, the age of laying hens, and egg and yolk weights were investigated. Various expressions of egg cholesterol values were also examined.

2.2.1. Materials and Methods

Ninety Single Comb White Leghorn hens, 22 wk of age, were housed in three floor pens with 30 birds per pen and were fed a wheat and soybean meal based laying hen ration containing 16% protein, 3.36% calcium, and 2,790 kcal/kg ME as described in Table 2.1. Eggs were collected on Days 0, 3, 6, 9, 12, 15, 82, and 180 starting from the 22nd wk. On each collection day, all eggs collected were pooled, and 15 eggs were randomly selected. Egg, yolk, and shell weights of each egg was measured, and three yolks were then pooled. About .2 g of pooled yolk samples were weighed, and saponified according to Abell et al. (1952) with minor modifications of incubation temperature at 50 C for 60 min (Sim and Bragg, 1977). The cholesterol was extracted and assayed by the enzymatic method as described previously (Jiang et al., 1990). Data were analyzed by a second-degree polynomial regression procedure to indicate the time trends of various parameters (Steel and Torrie, 1980).

2.2.3. Results and Discussion

As the laying sequence progressed during a 6-mo laying period starting from the 22nd wk, egg weight and yolk size in both absolute terms and as a percentage of the egg increased sharply during the first 2 wk and then slowly approached constant values by Day 180 (Figure 2.2). Previously, Bair et al. (1980) reported an increase of egg and yolk weights from the first egg to the fifth egg after onset of production. The present study showed that these trends were also true during the first 6 mo of production. On a relative basis, egg yolk as the percentage of the egg also increased in a similar pattern as the egg and yolk weights, indicating that egg yolk weight increased at a much faster rate than albumen. The rate of egg production during the second mo to the sixth decreased slightly but steadily.

The two common expressions of egg cholesterol values, mg/g yolk and mg/egg, exhibited opposite trends when plotted against the age of laying hens (Figure 2.2). In terms of mg/g yolk, the cholesterol value decreased sharply during the first 2 wk, and then slowly approached a constant value of 12.2 mg/g yolk by 6 mo of production. On the other hand, cholesterol levels expressed as mg/egg increased sharply during the first 2 wk of the laying period, and approached a constant value of 205 mg/egg.

The overall increase in mg/egg in spite of a decrease in the yolk cholesterol concentration (mg/g yolk) as the age of laying hens progressed indicated that the increase of egg yolk weight was greater than the reduction in yolk cholesterol concentration. Therefore, the determinative factor of cholesterol content of an egg was the weight of egg yolk. A smaller yolk contained less cholesterol than a large one.

It would not be appropriate, however, to conclude that smaller eggs are superior to larger ones due to their lower cholesterol content in mg/egg. Both yolk weight and egg weight were highly correlated with the age of laying hens. Smaller eggs have smaller yolks, and hence less cholesterol, but fewer nutrients, too. Because mg/egg and mg/g yolk units provide no information on the nutrient value of an egg, these two common expressions of egg cholesterol value could be very confusing to dietitians and consumers. A new term for egg cholesterol, milligram per gram egg (mg/g egg), was therefore adopted. Eggs laid during the first 2 wk of the production had a cholesterol value of 3.1 mg/g egg. Cholesterol content increased slightly to 3.4 for eggs collected on Day 180 (Figure 2.2). The new expression of egg cholesterol in mg/g egg might clear up some of the confusion caused by the egg producers' claim of "low cholesterol eggs" in the popular press. Providing that the determinative factor of cholesterol content of an egg was the yolk size and that smaller eggs were not superior to larger eggs when examined in mg/g egg, an ideal low cholesterol egg would be a large egg with a small yolk, i.e., an egg with a small proportion of yolk.

Table 2.1. Composition of laying hen diet

Ingredients	%
Ground wheat (13% CP)	66.5
Soybean meal (44% CP)	16.0
Tallow	4.0
Alfalfa meal (17% CP)	2.0
Ground limestone	8.0
Dicalcium phosphate	1.25
Iodized salt	.25
Layer vitamin-mineral mix[1]	2.0
Calculated composition	
CP, %	16.02
ME, kcal/kg	2790
Calcium, %	3.36
Total phosphorus, %	.64

[1] Layer vitamin-mineral mix supplied per kg of diet the following: vitamin A, 8000 I.U.; vitamin D_3, 1200 ICU; vitamin E, 5 I.U.; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin B_{12}, 10 μg; choline chloride, 100 mg; biotin, 100 μg; DL-methionine, 500mg; manganese sulfate, .4 g; zinc oxide, .1 g.

Table 2.2. The egg cholesterol values determined by the colorimetric, enzymatic, GC, and HPLC methods

Methods	Cholesterol values[1]	
	mg/g yolk	mg/egg[2]
Colorimetric	14.6 ± .5^{a}	242.8 ± 11.6^{a}
Enzymatic	12.3 ± .6^{b}	204.6 ± 13.9^{b}
GC	11.7 ± .4^{b}	194.6 ± 10.2^{b}
HPLC	11.7 ± .3^{b}	195.0 ± 10.5^{b}

^{a,b}: Means within the same column with no common superscripts are significantly different ($P<.01$).

[1] Mean ± SEM (n=5).

[2] Average egg weight (g): 60.38 ± 2.07; yolk weight (g): 16.67 ± .44.

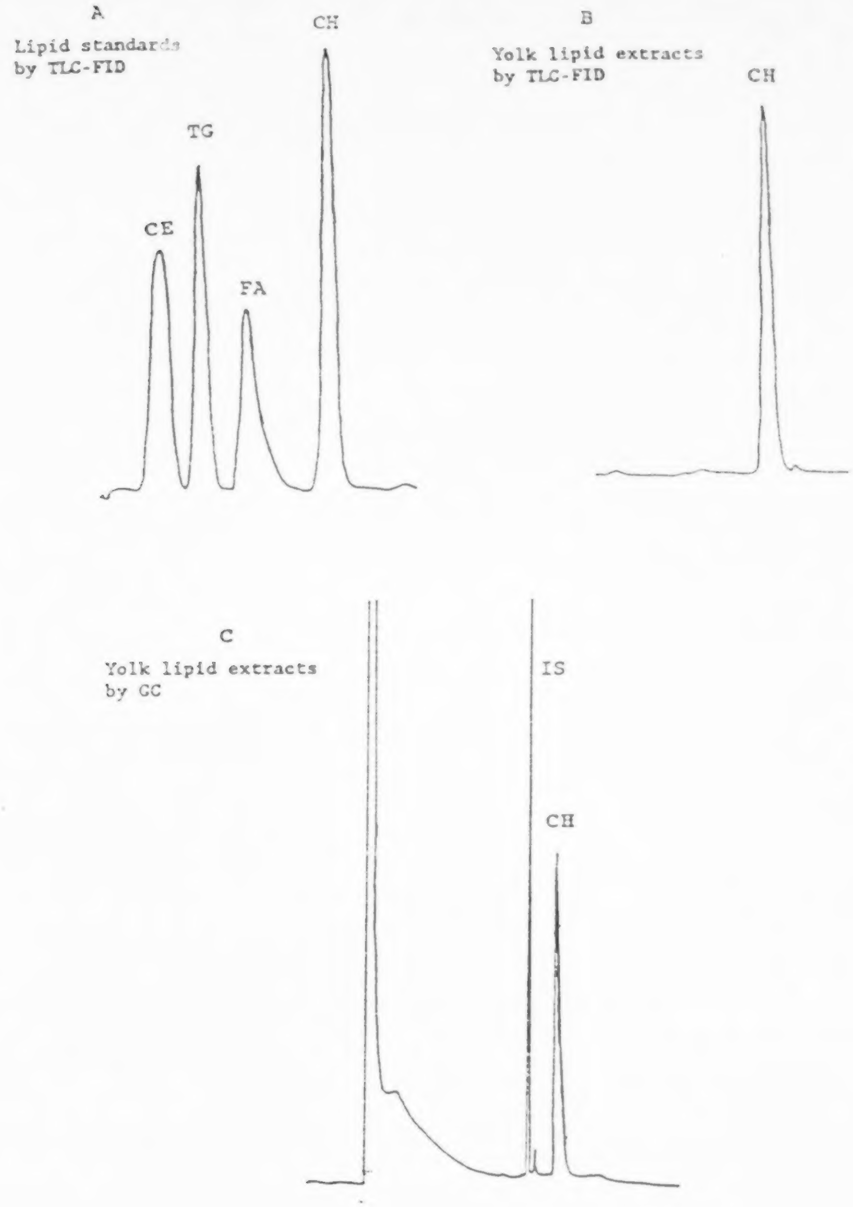
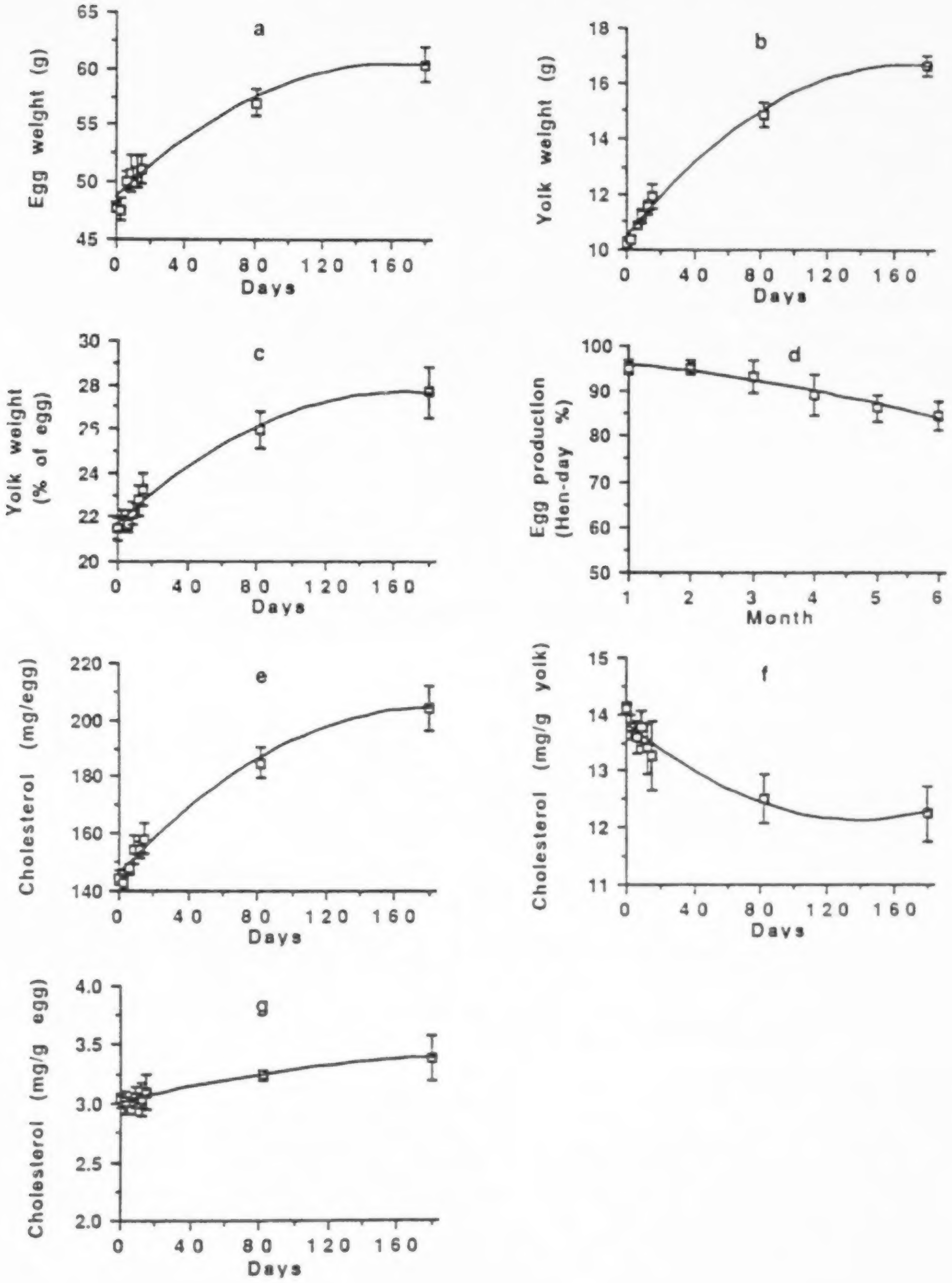


Figure 2.1. The chromatograms of lipid standards (Panel A) and egg yolk lipid extracts (Panel B) by the thin-layer chromatography-flame ionization detector (TLC-FID), and of yolk lipid extracts by gas chromatography (Panel C). No free fatty acids or triglycerides were detected in the yolk lipid extracts by either method. Abbreviations: CE: cholesterol oleate; TG: tripalmitin; FA: stearic acid; CH: cholesterol; IS: 5-α-cholestane.

Figure 2.2. Egg weight, yolk size in both absolute weight and percentage of egg, rate of egg production, and cholesterol values in milligram per gram of yolk, milligram per egg, or milligram per gram of egg as the laying sequence progressed from the 22nd wk (Day 0). Each point was the mean (± SD) of five pooled samples of three eggs each, except for egg production rate where each point was the mean (± SD) of three pens of 30 laying hens each. The second-degree polynomial regression equations and R^2 values for graphs a to g were: a) $Y = 48.603 + .1438X - .000438X^2$, $R^2 = .965$; b) $Y = 10.576 + .0736X - .000219X^2$, $R^2 = .990$; c) $Y = 21.641 + .0721X - .000216X^2$, $R^2 = .981$; d) $Y = 97.130 - 1.0282X - .191079X^2$, $R^2 = .960$; e) $Y = 145.56 + .6429X - .001768X^2$, $R^2 = .985$; f) $Y = 13.840 - .0246X + .000088X^2$, $R^2 = .938$; g) $Y = 3.001 + .0036X - .000008X^2$, $R^2 = .947$.


a
Egg weight (g)
65
60
55
50
45
0
40
80
120
160
Days
b
Yolk weight (g)
18
16
14
12
10
0
40
80
120
160
Days
c
Yolk weight
(% of egg)
30
28
26
24
22
20
0
40
80
120
160
Days
d
Egg production
(Hen-day %)
100
90
80
70
60
50
1
2
3
4
5
6
Month
e
Cholesterol (mg/egg)
220
200
180
160
140
0
40
80
120
160
Days
f
Cholesterol (mg/g yolk)
15
14
13
12
11
0
40
80
120
160
Days
g
Cholesterol (mg/g egg)
4.0
3.5
3.0
2.5
2.0
0
40
80
120
160
Days

2.3 References

Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall, 1952. A simplified method for the estimation of total cholesterol in serum. J. Biol. Chem. 195:357-366.

Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475.

Bair, C. W., W. W. Marion, and D. K. Hotchkiss, 1980. Relationship of yolk cholesterol and onset of egg production. Poultry Sci. 59:666-668.

Bartov, I., S. Bornstein, and P. Budowski, 1971. Variability of cholesterol concentration in plasma and egg yolks on hens and evaluation of the effect of some dietary oils. Poultry Sci. 50:1357-1364.

Beyer, R. S., and L. S. Jensen, 1989. Overestimation of cholesterol content of eggs. J. Agric. Food Chem. 37:917-920.

Bohac, C. E., K. S. Rhee, H. R. Cross, and K. Ono, 1988. Assessment of methodologies for colorimetric cholesterol assay of meats. J. Food Sci. 53:1642-1644.

Cotterill, O. J., W. W. Marion, and E. C. Naber, 1977. A nutrient re-evaluation of shell eggs. Poultry Sci. 56:1927-1934.

Cunningham, D. L., W. F. Krueger, R. C. Fanguy, and J. W. Bradley, 1974. Preliminary results of bidirectional selection for yolk cholesterol level in laying hens. Poultry Sci. 53:384-391.

Cunningham, F. E., 1977. Composition of Araucana eggs. Poultry Sci. 56:463-467.

De La Huerga, J., and J. C. Sherrick, 1972. The measurement of cholesterol in serum: reference method. Annals of Clinical Laboratory Sci. 2(5):360-366.

Jiang, Z., G. Cherian, F. E. Robinson, and J. S. Sim, 1990. Effect of feeding cholesterol to laying hens and chicks on cholesterol metabolism in pre and post-hatch chicks. Poultry Sci. 69:1694-1701.

Jiang, Z., M. Fenton, and J. S. Sim, 1991. Comparison of four different methods for egg cholesterol determination. Poultry Sci. 70:1015-1019.

Naber, E. C., 1989. What is the real cholesterol content of an egg? Poultry Sci. 68(Suppl. 1):103 (Abstr).

Nichols, E. L., W. W. Marion, and S. L. Balloun, 1963. Effect of egg yolk size on cholesterol concentration. Proc. Soc. Exp. Biol. Med. 112:378-380.

Nix, D. F., E. J. Thornton, K. W. Washburn, and H. L. Marks, 1974. The influence of molting on yolk cholesterol level. Poultry Sci. 53:412-414.

Sim, J. S., and D. B. Bragg, 1977. Effect of dietary factors on serum and egg yolk cholesterol levels of laying hens. Poultry Sci. 56:1616-1621.

Spencer, J. V., W. A. Becker, L. W. Mirosh, and J. A. Verstrate, 1978. Effect of fertilization and age of hen on the cholesterol content of chicken egg yolk. Poultry Sci. 57:261-264.

Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill, New York, NY.

Turk, D. E., and B. D. Barnett, 1970. Cholesterol content of market eggs. Poultry Sci. 49:1445 (Abstr.).

Turk, D. E., and B. D. Barnett, 1971. Cholesterol content of market eggs. Poultry Sci. 50:1980-1982.

USDA, 1976. Composition of Foods, Dairy and Egg Products, Raw-Processed-Prepared. USDA Agriculture Handbook 8-1. Washington, D. C..

USDA, 1989. Composition of Foods, Dairy and Egg Products, Raw-Processed-Prepared. USDA Agriculture Handbook 8-1. Washington, D. C..

Washburn, K. W., and D. F. Nix, 1974. Genetic basis of yolk cholesterol content. Poultry Sci. 53:109-115.

Chapter 3. Comparison of the Cholesterolemic Effects of Egg Yolk Powder Versus Crystalline Cholesterol in Rats[1]

3.1. Introduction

Public concern over cholesterol content of eggs is certainly one of the major factors causing the continuous decrease in per capita egg consumption during the last four decades. The relationship between egg consumption and cholesterol metabolism, however, still has not been clarified (Oster, 1982; Connor, 1982; Edington et al., 1989). Early works reported that egg yolk powder produced higher plasma cholesterol levels than did the same or much larger amounts of crystalline cholesterol (Messinger et al., 1950; Connor et al., 1961; Splitter et al., 1968). Recent studies demonstrated that plasma total cholesterol level was lower in chicks fed egg yolk as a cholesterol source than in those fed the same amount of crystalline cholesterol (Sim et al., 1980; Kim and Han, 1985). In those studies, the difference of dietary protein content caused by egg yolk supplement was balanced by plant proteins such as soybean meal. In general, dietary plant proteins are hypocholesterolemic as compared to animal proteins such as casein (Carroll, 1983). Furthermore, egg yolk contains about 60% lipids (dry matter basis), mainly as triglycerides and phospholipids. Both the quantity and the fatty acid compositions of dietary lipids influence cholesterol metabolism (McNamara, 1987).

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The objective of the present research was to examine the influence of dietary egg yolk powder versus crystalline cholesterol on the plasma, lipoprotein, and tissue cholesterol levels when the protein and lipid contents of the control diet were balanced by supplementing casein and lard, respectively. Lard was chosen because it is of animal origin and has a fatty acid profile similar to egg yolk lipids (Table 3.1).

3.2. Materials and Methods

Two groups of eight male Sprague-Dawley rats 4 wk of age were housed individually in wire cages at 21 C and a 12-hr light/dark cycle. Two isonitrogenous test diets were prepared (Table 3.2). One contained 18.7% spray dried egg yolk powder (YP, Highland Produce Ltd., Two Hills, Alberta, Canada, T0B 4K0), and the other was balanced for cholesterol, fat, and protein contents by adding crystalline cholesterol (Sigma Chemical Company, St. Louis, MO 63178), lard, and casein (CN, Teklad, Madison, WI 53711). The calculated cholesterol contents of the diets were 5.01 and 5.00 g cholesterol per kg feed for YP and CN diet, respectively. Feed and water were provided ad libitum. Body weight and feed consumption were measured weekly.

At the end of the four-week feeding trial, rats were fasted overnight and decapitated. Blood was collected into heparin-Na^+ coated tubes. Plasma was obtained by centrifugation at 2,000 x g for 30 min. The high-density lipoprotein fraction was prepared by precipitating and removing of very-low density and low-density lipoprotein (VLDL and LDL) with dextran sulfate-Mg^{2+} (Warnick et al., 1982). Liver and heart tissues were removed, weighed, and stored at -20 C until lipid analysis.

Cholesterol concentrations of plasma, HDL fraction, liver and heart tissues were determined enzymatically as described previously (Jiang et al., 1990). Differences between treatment means were tested by the Student's t-test (Steel and Torrie, 1980).

3.3. Results and Discussion

No significant difference in body weight gain nor feed consumption was found between the two groups of rats fed either YP or CN in the present study (Table 3.3). Neither were plasma total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels significantly influenced by these dietary treatments (Table 3.3). Numerically, HDL-C levels of rats fed YP diet were more than 10% higher than values in those fed CN diet.

Early studies with human subjects indicated that dietary egg yolk was more hypercholesterolemic than crystalline cholesterol (Messinger et al., 1950; Cook et al., 1956). This was attributed to either the low absorption of crystalline cholesterol when it was fed without oil (Sylven and Borgstrom, 1968), or the hypocholesterolemic action of polyunsaturated fatty acids (Paul et al., 1980) which were included in the crystalline cholesterol diet. Other studies, however, revealed that dietary egg yolks were less cholesterolemic than crystalline cholesterol in chickens (Sim et al., 1980; Kim and Han, 1985). Bartov et al. (1973) also found that feeding egg yolks to female rats produced lower plasma cholesterol levels than did the same amount of crystalline cholesterol. In the present study, rats fed YP diet had only numerically lower plasma TC and higher HDL-C.

The difference of male and female rats in the response of plasma cholesterol to dietary treatments might explain the discrepancy between our results and those of Bartov et al. (1973). The blood cholesterol levels of female rats were reported to be more responsive to dietary manipulation than those of males (Terpstra et al., 1982).

However, both free and total cholesterol contents of liver, but not heart tissues, of rats fed YP diet was significantly lower than those fed CN (Table 3.3). The two test diets contained same the level of lipids and had similar fatty acid composition, ruling out any effect of fatty acid profile of the diets. Two factors of the yolk powder diet could be responsible for the difference in the liver cholesterol content. The first is egg yolk lipids. The YP diet contained 3.3% yolk phospholipids in addition to about 7% yolk neutral oil (triglycerides). Dietary phospholipids from egg yolks have been shown to specifically increase plasma HDL-C in guinea pigs (O'Brien and Corrigan, 1988). The second factor might be the protein sources per se. Yolk protein might influence cholesterol metabolism in rats rather differently from casein (Carrol, 1983). Further studies are thus needed to understand the effects of yolk components such as yolk neutral oil and lecithin on plasma and HDL cholesterol levels.

Table 3.1. Major fatty acids of test diets containing either yolk powder (YP) or casein (CN)

Fatty acid	Yolk powder diet	Casein diet
	----- (% of total fatty acids) -----	
C16:0	2.9	2.3
C18:0	9.7	16.2
C16:1(n-7 & n-9)	2.9	2.3
C18:1(n-7 & n-9)	47.5	45.2
C18:2(n-6)	11.7	11.0
C18:3(n-3)	.7	.7
Σ Saturated	12.6	18.5
Σ MUFA[1]	50.4	47.5
Σ PUFA[2]	14.5	11.7

[1] monounsaturated fatty acids.

[2] polyunsaturated fatty acids.

Table 3.2. Nutrient composition of yolk powder (YP) and casein diet (CN)

Nutrient[1]	YP diet	CN diet
	(g/kg Feed)	
Casein, high protein (87% CP)	169.6	247.2
Glucose, monohydrate	190.5	190.5
Corn starch	350.8	350.8
DL-methionine	2.4	2.4
Choline chloride	1.8	1.8
Inositol	5.6	5.6
Vitamin Mix, A.O.A.C.	9.3	9.3
Mineral Mix, Bernhart-Tomarelli	46.8	46.8
Cellulose	36.2	45.6
Cholesterol	0	5.0
Yolk powder[2]	187.0	0
Lard	0	95.0

[1] All nutrients, except choline chloride, cholesterol (Sigma Chemical Company, St. Louis, MO) and yolk powder (Highland Produce Ltd., Two Hills, Alberta), were from Teklad, Madison, WI.

[2] The determined analyses of the yolk powder were: DM, 97.78%; CP, 36.10%; total lipid (including cholesterol), 53.40%; cholesterol, 2.68%. At 187 g/kg, it provided 99.86 g total lipid, which included 5.01 g cholesterol per kg of YP diet.

Table 3.3. The performance, and plasma, HDL, liver, and heart cholesterol levels of rats fed yolk powder diet or casein diet

Variables	n	Diet Yolk powder (YP)	 Casein(CN)
Performance			
Feed consumed (g/4 wk)	8	517.5 ± 25.8	580.1 ± 53.3
Body weight gain (g/4 wk)	8	194.1 ± 25.8	203.5 ± 18.8
Cholesterol			
Plasma TC (mg/100mL)[1]	8	84.52 ± 12.80	89.12 ± 11.54
HDL-C (mg/100mL)[1]	8	42.62 ± 6.97	38.10 ± 7.10
HDL-C/TC ratio		.507	.438
Liver tissue			
Total lipids (% of tissue)	8	17.44 ± 3.20	19.40 ± 2.10
Free cholesterol (mg/g tissue)	8	4.76 ± .94	7.43 ± 1.26*
Total cholesterol (mg/g tissue)	8	13.77 ± 2.15	16.40 ± 2.25*
Heart tissue			
Total lipids (% of tissue)	8	3.48 ± .32	3.56 ± .27
Free cholesterol (mg/g tissue)	8	2.37 ± .51	2.80 ± .29
Total cholesterol (mg/g tissue)	8	3.41 ± .67	3.79 ± .63

[1] TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol. Data were presented as Mean ± SD.

* P < .05.

3.4. Reference

Bartov, I., R. Reiser, and G. R. Henderson, 1973. Hypercholesterolemic effect in the female rat of egg yolk versus crystalline cholesterol dissolved in lard. J. Nutr. 103:1400-1405.

Connor, W. E., 1982. Letters to the editor. Am. J. Clin. Nutr. 36:1261-1262.

Connor, W. E., R. E. Hodges, and R. E. Bleiler, 1961. The serum lipids in men receiving high cholesterol and cholesterol-free diet. J. Clin. Invest. 40:894-902.

Carroll, K. K., 1983. Dietary proteins and amino acids-their effects on cholesterol metabolism. Pages 9-17 in: Animal and Vegetable Proteins in Lipid Metabolism and Atherosclerosis. M. J. Gibney and D. Kritchevsky, ed. Alan R. Liss, Inc., New York, NY.

Cook, R. P., D. C. Edwards, and C. Riddell, 1956. Cholesterol Metabolism. 7. Cholesterol absorption and excretion in man. Biochem. J. 62:225-234.

Edington, J. D., M. Geekie, R. Carter, L. Benfield, M. Ball, and J. Mann, 1989. Serum lipid response to dietary cholesterol in subjects fed a low-fat, high-fiber diet. Am. J. Clin. Nutr. 50:58-62.

Jiang, Z., G. Cherian, F. E. Robinson, and J. S. Sim, 1990. Effect of feeding cholesterol to laying hens and chicks on cholesterol metabolism in pre and post-hatch chicks. Poultry Science 69:1694-1701.

Kim, K. N., and I. K. Han, 1985. Effects of different dietary sources of cholesterol, protein and fiber on lipid metabolism in broiler chicks. Korean J. Animal Sci. 27(6):362-366.

McNamara, D. J., 1987. Effects of fat-modified diets on cholesterol and lipoprotein metabolism. Ann. Rev. Nutr. 7:273-290.

Messinger, W. J., Y. Porosowska, and J. M. Steele, 1950. Effect of feeding egg yolk and cholesterol on serum cholesterol levels. Arch. Intern. Med. 86:189-195.

O'Brien, B. C. and S. M. Corrigan, 1988. Influence of dietary soybean and egg lecithins on lipid responses in cholesterol-fed guinea pigs. Lipids 23:647-650.

Oster, K. A., 1982. Letters to the editor. Am. J. Clin. Nutr. 36:1259-1261.

Paul, R., C. S. Ramesha, and J. Ganguly, 1980. On the mechanism of hypocholesterolemic effects of polyunsaturated lipids. Adv. Lipid

Res. 17:155-171.

Sim, J. S., W. D. Kitts, and D. B. Bragg, 1980. Effect of dietary egg yolk on serum cholesterol levels of White Leghorn cockerels. Poultry Sci. 59:1812-1817.

Splitter, S. D., G. D. Michaels, G. Schlierf, P. D. S. Wood and L. W. Kinsell, 1968. Evaluation of the effects of egg yolk lipids upon plasma lipids in human subjects. Metabolism 17:1129-1135.

Steel, R. G. D., and J. H. Torrie, 1980. in: Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill, New York, NY.

Sylven, C., and B. Borgstrom, 1968. Absorption and lymphatic transport of cholesterol in the rat. J. Lipid Res. 9:596-601.

Terpstra, A. H. M., G. van Tintelen, and C. E. West, 1982. The effect of semipurified diets containing different proportions of either casein or soybean protein on the concentration of cholesterol in whole serum, serum lipoproteins and liver in male and female rats. Atherosclerosis 42:85-95.

Warnick, G. R., J. Benderson, and J. J. Albers, 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high density lipoprotein cholesterol. Clin. Chem. 28:1379-1388.

Chapter 4. Effects of Egg Yolk Neutral and Phospholipids on Plasma and Tissue Cholesterol Levels in Rats

4.1. Cholesterolemic Effects of Egg Yolk Neutral Oil

4.1.1. Introduction

The effects of cholesterol intake, mainly as egg consumption, on plasma and lipoprotein cholesterol levels have still not been clarified (Oster, 1982; Connor and Connor, 1983; Edington et al., 1987; Stehbens, 1989; Steinberg, 1989). In most studies, whole eggs or egg yolks were used as the source of dietary cholesterol. The controversial results regarding the cholesterolemic action of egg consumption can partly be attributed to the complex influences of egg components other than cholesterol per se, as demonstrated in the previous chapter and other studies (Bartov et al., 1973; Kim and Han, 1985). Egg yolk triglycerides, for example, might also influence cholesterol metabolism. Egg yolk lipids constitute about 60% of egg yolk dry matter, and 70% of yolk lipids are neutral oil (triglycerides). Yolk neutral oil consists of more than 50% monounsaturated fatty acids, and 11% polyunsaturated fatty acids (Powrie and Nakai, 1986). Recently, it has been reported that vegetable oils rich in monounsaturated fatty acids, such as safflower and olive oil, are also hypocholesterolemic (Mattson and Grundy, 1985; Mensink and Katan, 1989). When saturated fats were substituted by these oils, plasma total (TC) and low-density lipoprotein cholesterol (LDL-C) levels were reduced. But unlike dietary n-6 polyunsaturates such as linoleic acid, dietary monounsaturates did not reduce HDL-C level (Mattson and Grundy, 1985;

Baggio et al., 1988; Mensink and Katan, 1989). Egg yolk neutral oil, with more than 50% monounsaturated fatty acids, may thus play a role in influencing the cholesterol metabolism when whole egg or egg yolk is consumed. In addition, egg yolk lipids might be useful in preparation of infant formula to achieve a fatty acid profile analogous to that of human milk (Tokarska and Clandinin, 1985). Thus, it is of interest to understand the cholesterolemic property of the dietary egg yolk oil. Little, if any, information regarding this subject is available.

The present study was designed to investigate the cholesterolemic effects of dietary egg yolk neutral oil in rats by comparing with oils of various degrees of saturation

4.1.2. Materials and Methods

Animals and diets

Weanling male Sprague-Dawley rats, weighing about 105 grams, were obtained from the Bioscience Animal Services of the University of Alberta, Edmonton, Alberta, Canada. Rats were housed individually in wire-cages in a room with the temperature at 21 C and 12-hr alternating light-and-dark period. Water and feed were provided ad libitum. The feed consumption and body weight were measured twice every week.

All rats were fed Wayne Rodent Blox (Continental Grain Company, Chicago, IL) in the first wk. They were then randomly divided into four groups with seven rats in each group. The composition of test diets is shown in Table 4.1. Dietary cholesterol level was .2%. Four types of lipids were tested. They were: saturated (SAFA, coconut oil/safflower oil = 4/1); egg yolk neutral oil (YNO); lard (LARD); and polyunsaturated

(PUFA, coconut oil/safflower oil = 1/4). Mixtures of coconut oil and safflower oil were used to prepare SAFA and PUFA to achieve a wide range of polyunsaturated to saturated (P/S) ratios. The fatty acid profiles of the test diets were determined by gas chromatographic (GC) method as described elsewhere (Jiang et al., 1991) and were presented in Table 4.2. Egg yolk neutral oil was prepared from fresh egg yolks by an ethanolic extraction procedure developed in our laboratory (Sim, Canada Patent No. 612411). Its major component was triglycerides (>98%) with 1.06% cholesterol as determined by the GC and thin-layer chromatographic technique (Iatroscan TH-10, Iatron Labs, Japan). Coconut oil, safflower oil and lard were purchased from United States Biochemical Corporation, Cleveland, Ohio. Cholesterol (Sigma Chemical Co., St. Louis, MO) was dissolved in warm oils by adequate stirring before being mixed with basal preparation to achieve a .2% dietary level.

Sample preparation and analysis

At the end of 4-week feeding period, rats were fasted overnight. Blood was collected into Vacutainers (Becton Dickinson Canada Inc., Mississagua, Ontario, Canada) by heart puncture when animals were under haloothane anaesthesia (Laboratoires Ayerst, Montreal, Canada).

Plasma was obtained by centrifugation at 1500xg for 30 min. High-density lipoprotein (HDL) fraction was prepared after precipitation and removal of LDL and VLDL with phosphotungstic acid-$MgCl_2$ (Burstein et al., 1970). Cholesterol content was assayed by the enzymatic method of Allain et al. (1974) and Ho and Dupont (1981). In brief, an enzyme solution containing cholesterol oxidase, cholesterol esterase, and peroxidase (Sigma Chemical Ltd., St. Louis, MO) was prepared for total cholesterol assay. Samples of whole plasma and HDL fraction were added directly to enzyme solution for color development. Cholesterol standard stock (Sigma Chemical Co., St. Louis, MO) was used to construct the standard absorbance curve.

Statistics

Aanalysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were employed to analyze the data (Steel and Torrie, 1980).

4.1.3. Results and Discussion

The lipid content of experimental diets was designed to resemble the current diet consumed by North Americans, with lipid contributing about 36% of total energy. The P/S ratios of these diets ranged from .14 to 1.34, well bracketing the recommended value (P/S = 1.0) by the American Heart Association (1988). The YNO diet had a fatty acid profile very similar to that of the LARD diet. In the present study, the type of dietary fats did not influence the apparent performance of the rat in terms of body weight gain, feed consumption, or feed conversion efficiency (Table 4.3).

The plasma total cholesterol (TC) and HDL cholesterol (HDL-C) levels of rats at the end of the feeding trial were shown in Table 4.4. Rats fed SAFA diet had significantly higher plasma TC as well as HDL-C than those fed YNO, LARD, or PUFA. This corroborated previous results of many studies that dietary saturated fats were hypercholesterolemic when compared to monounsaturated or polyunsaturated ones (Keys et al., 1965; Hegsted et al., 1965; Glueck, 1979).

There was no significant differences in plasma TC or HDL-C among rats fed YNO, LARD, or PUFA, although numerically PUFA diet resulted in the lowest level of both TC and HDL-C in rats among all dietary treatments. Egg yolk neutral oil and lard are animal fats. Dietary fats of animal origin generally contain 45-55% monounsaturated and 10 to 15% PUFA (Kuksis, 1978). As monounsaturated fatty acids had been previously considered as "neutral" in influencing the plasma cholesterol level (Keys et al, 1965; Hegsted et al., 1965), the animal fats, with major proportion of monounsaturated fatty acids and low P/S ratios, have been traditionally classified as saturated, and a hypercholesterolemic property of dietary animal fats has been frequently implied. Recent studies have demonstrated that monounsaturated fats of vegetable origin, in particular, oleic acid-rich safflower oil and olive oil, are hypocholesterolemic (Mattson and Grundy, 1985; Baggio et al., 1988; Mensink and Katan, 1989). The traditional classification of animal fats, which contain more than 50% monounsaturated, as "saturated" might thus be inappropriate and could be one of the contributory factors causing the controversial claims regarding the cholesterolemic action of dietary "saturated" fats (Reiser, 1973). Indeed, dietary beef fat, although commonly classified as saturated, did not exhibit hypercholesterolemic properties as it would be expected (Reiser et al., 1985). The high content of monounsaturated fatty acids in these animal fats might explain the observed phenomena.

The magnitude of cholesterol-lowering effects of MUFA, however, is still in dispute (Becker et al., 1983; Mattson and Grundy, 1985). Our results demonstrated that dietary yolk neutral oil and lard were, statistically, as effective as n-6 polyunsaturated fats in lowering plasma TC in rats. Numerically, however, the PUFA diet reduced both plasma TC and HDL-C further by about 11% and 12%, respectively, indicating that in rats dietary polyunsaturates might be more hypercholesterolemic than monounsaturates. In some clinical studies, dietary oleic acid was reported to be less effective in lowering plasma cholesterol levels than was linoleic acid (Becker et al., 1983).

4.2. Cholesterolemic Effects of Egg Yolk Phospholipids in Rats

4.2.1. Introduction

The other major component of yolk lipids is yolk phospholipids. Yolk phospholipids constitute about 30% of yolk total lipids. The majority of yolk phospholipids is phosphatidylcholine (PC, 70%) and phosphatidylethanolamine (PE, 24%) (Noble, 1987).

Phospholipids, particularly PC from soybeans, or as commonly referred to as soy lecithin, have been reported to be hypocholesterolemic in various species of laboratory animals (Byers and Friedman 1960; Howard et al. 1971; Samochowiec et al. 1976; Wong et al. 1980; Hunt and Duncan 1985; Jimenez et al. 1990) and human subjects (Childs et al. 1981; Leotta and Fusco 1985; Kesaniemi and Grundy 1986). Intake of soy lecithin increased elimination of cholesterol from the animal body via fecal excretion of neutral sterols (Greten et al. 1980; Hollander and Morgan 1980; O'Mullane and Hawthrone 1982; Kesaniemi and Grundy 1986). It was not clear, however, whether the reported hypocholesterolemic effects of soy lecithin were due to the lecithin structure *per se*, or were mediated through linoleic acid (LA). Soy lecithin is rich in LA of which the hypocholesterolemic effect is well documented (Paul et al. 1980).

Egg phospholipids, or egg lecithin, differ markedly from soy lecithin in fatty acid composition. The fatty acid chains of soy lecithin are highly polyunsaturated due to high contents of LA and linolenic acid (LNA), while egg lecithin contains mainly palmitic and oleic acids (Noble 1987). In contrast to the abundant information on soy lecithin, little is known about the cholesterolemic action of dietary egg lecithin (O'Brien and Corrigan 1988). Early intravenous infusion studies revealed similar anti-atherogenic effect of phospholipids extracted from both plant and animal tissues, despite their marked difference in the degree of fatty acid unsaturation (Byers and Friedman 1960). On the other hand, saturated (hydrogenated) soy lecithin was reported to be less effective in resorption of implanted cholesterol than soy lecithin in its natural form (Adams and Morgan, 1967), indicating that the effects of lecithin on cholesterol metabolism were modified by its fatty acid composition.

The objective of this study was to examine 1) whether dietary lecithin per se had independent, systemic effects on cholesterol metabolism when compared with triglycerides of similar fatty acid composition; and 2) how the two forms of lecithin, i.e., more saturated egg yolk lecithin versus unsaturated soy lecithin, influenced the cholesterol metabolism in rats.

4.2.2. Materials and Methods

Animals and diets

Male weanling Sprague-Dawley rats (Bioscience Animal Services of

University of Alberts), 4 wk of age, were housed individually in metabolic wire cages at 21 C with a 12-h light/dark cycle. After feeding of a commercial diet ad libitum (Wayne Rodent Blox, Continental Grain Company, Chicago, IL) for three days, they were divided into four groups with eight rats each, in such a manner that the average body weight of all groups was similar. Crude egg yolk lecithin (EL) was prepared from fresh egg yolk by an ethanolic extraction system developed in our lab (Sim, 1991). The egg lecithin obtained contained 90.7% phospholipid (PL), 6.7% cholesterol, and 2.5% triglycerides (TG). Soy lecithin (SL) was purchased from Canada Packers Ltd. (Toronto, Canada). It contained 77.4% PL and 21.6% TG. Lard (LD) and soybean oil (SO), which have similar fatty acid profiles as egg lecithin and soy lecithin, respectively, were used as the dietary triglyceride counterparts. Seven percent EL, SL, or 5% LD, and SO were mixed with a semi-synthetic basal diet to give four test diets (Table 4.5). The 7% of EL and SL provided similar amounts of fatty acids present in 5% LD or SO. Water and feed were provided ad libitum. Feed consumption and body weight gain were measured weekly. Feces were collected during the fourth wk, using Fe_2O_3 as a marker (McDonald et al. 1981).

Sample preparation and analysis

At the end of the 4-wk feeding trial, rats were fasted overnight. They were sacrificed by decapitation and blood was collected into heparin-Na^+ coated tubes. Rat livers were removed, blotted dry on towel paper, weighed, and stored at -20 C. Plasma was separated by centrifugation at 2,000 x g for 30 min. The high-density lipoprotein (HDL) fraction was prepared by precipitation and removal of very-low-density and low-density lipoproteins (VLDL and LDL) with dextran sulfate-Mg^{2+} (Warnick et al. 1982). Cholesterol of plasma and HDL fraction were measured using a commercial diagnostic kit (Procedure #352, Sigma Diagnostic, St. Louis, MO).

A portion (1.0 g) of median lobe of rat liver was ground with a mixture of CH_3Cl and CH_3OH (2/1, v/v) (Folch et al. 1957) and the total lipid content of liver was determined gravimetrically. Contents of free and esterified cholesterol of rat liver were assayed by an enzymatic method (Carlson and Goldfarb 1977).

Rat feces were dried at 110 C overnight, and ground. The fecal neutral sterols were extracted according to Miettinen et al. (1965), with 5-α-cholestane added as the internal standard. The unsaponifiable extracts were directly injected on a DB-17 capillary column (J & W Scientific, Folsom, CA) in a Varian Model 3700 (Varian Associates, Inc., Walnut creek, CA) gas chromatograph. The column temperature was programmed to be initially at 230 C, increased to 280 C at a rate of 5 C/min, and held at 280 C for 13 min. The flame ionizing detector was set at 280 C.

Statistical analysis

Treatment means were subjected to one-way analysis of variance. When treatment effects were significant ($P < .05$), means were further analyzed by Duncan's multiple range test (Steel and Torrie 1980), with $P < .05$ as the criterion of significance.

4.2.3. Results

Fatty acid profiles of diets and rat performance

The fatty acid compositions of test diets were analyzed by gas chromatography (Metcalfe et al. 1961) and presented in Table 4.6. The fatty acid profile of EL was similar to that of LD, and so was SL to SO. EL and LD diets were much more saturated than SL and SO diets. Neither the body weight gain, nor the daily feed intake of rats were affected by dietary treatment (Table 4.7).

Plasma and lipoprotein lipids

Plasma total cholesterol (TC) was significantly lower in rats fed SL or SO than in those fed EL or LD (Figure 4.1). Egg lecithin feeding elevated high-density lipoprotein (HDL-C) significantly over soy lecithin, lard, or soybean oil feeding (Fig. 4.1). Consequently, lecithin feeding, regardless of the difference in degree of fatty acid saturation, resulted in similar HDL-C/TC ratios, which were significantly higher than in those fed the two triglyceride diets.

Liver lipid contents

As shown in Table 4.8, the two lecithin diets (EL and SL) resulted in significantly lower hepatic total lipid and total cholesterol than the two triglyceride diets (LD and SO). Further accumulation of liver lipids was observed in rats fed the SO diet over those fed the lard diet. The liver size of rats, however, was not affected by diets.

Neutral sterol excretion

Rats fed SL excreted significantly higher amount of total neutral sterols via feces than those fed the three other diets (Fig. 4.2). This was mainly due to the increased excretion of coprostanol. No significant difference was detected among the neutral sterols excreted by rats fed EL, LD, or SO.

4.2.4 Discussion

This study clearly demonstrated that dietary lecithin of both plant and animal origins had specific, systemic effects on cholesterol homeostasis in rats, which were different from those of dietary TG of similar fatty acid composition.

Although the anti-atherogenic property of dietary lecithin has been reported in both laboratory animals and human subjects, it was suggested that these observations were artifacts of other dietary changes or the linolenic acid present in lecithin preparations (Knuiman et al. 1989). To examine whether dietary lecithin *per se* has specific influences on plasma and lipoprotein cholesterol levels and cholesterol homeostasis, two forms dietary lecithin, egg yolk lecithin (EL) and soybean lecithin (SL), were compared to dietary TG, lard and soybean oil. The fatty acid compositions and polyunsaturated to saturated (P/S) ratios of lard and soybean oil were similar to those of egg lecithin and soy lecithin, respectively (Table 4.6). Therefore, when lecithin regimes were compared to triglyceride ones, possible effects of fatty acids could be minimized. This study clearly demonstrated that dietary lecithin did affect cholesterol homeostasis of rats in a way that was different from their dietary triglyceride counterparts. Firstly, significantly higher HDL-C/TC ratios were generated by EL and SL regimes when compared to LD and SO feeding. The HDL-C level was suggested to represent the reverse transport of cholesterol (Tall and Small 1980) and HDL-C/TC ratio was reported to be a better parameter than TC alone in assessing the atherosclerotic risks (Kannel and Gordon 1982; Castelli et al. 1986). The two lecithin diets therefore resulted in favorable lipoprotein cholesterol profiles in terms of HDL-C/TC ratio when compared to the TG diets. Furthermore, the lecithin diets also resulted in lesser liver total lipids and cholesterol than the TG diets, regardless of the marked difference in fatty acid compositions of EL and SL. While the unsaturation of TG resulted in further accumulation of hepatic lipids in SO fed rats, the degree of saturation of dietary lecithin did not affect rat hepatic lipid contents, indicating again the different dietary effects of lecithin and TG on lipid metabolism.

The two forms of dietary lecithin generated higher HDL-C/TC ratios in quite different ways. The high HDL-C/TC ratio of SL fed rats was the result of a lowered TC value. The precise mechanism of the hypocholesterolemic property of dietary soy lecithin is not unequivocal (Greten et al. 1980; Hollander and Morgan 1980; Jimenez et al. 1990). In the present study, dietary soy lecithin was found to increase the removal of cholesterol metabolites via feces. A significantly larger amount of neutral sterols, particularly coprostanol, were excreted by rats fed the SL diet (Table 4.9), indicating a reduction of intestinal absorption and/or reabsorption of cholesterol. This corroborated previous reports that intake of soybean lecithin resulted in the increase of excretion of neutral sterols in both experimental animals and human subjects (Greten et al. 1980; Hollander and Morgan 1980; O'Mullane and Hawthrone 1982;

Kesaniemi and Grundy 1986). A recent study, however, reported that the excretion of neutral sterols was not increased by dietary soy lecithin in hypercholesterolemic rats (Jimenez et al. 1990). A much lower level of dietary soy lecithin was used by Jimenez et al. (1990) than those used in this and other studies (Greten et al. 1980; Hollander and Morgan 1980; O'Mullane and Hawthrone 1982; Kesaniemi and Grundy 1986), which might explain the discrepancy.

Then the question arises as to whether the increased excretion of neutral sterols is due to soy lecithin *per se*, or the plant sterol contaminants of the soy lecithin preparation, as studies in men have shown that some of the plant sterols diminish intestinal absorption of cholesterol (Farquhar and Sokolow 1958; Lees et al. 1977). The following two points might provide an answer to the question. Firstly, it has been reported that plant sterols, such as stigmasterol and sitosterol, the main sterols of soy lecithin and soybean oil, did not affect the intestinal absorption of cholesterol in rats (Hollander and Morgan 1980). Secondly, the plant sterol contents of SL and SO diets were only marginally different. As determined by the GC method described in the Material and Methods section, soy lecithin contained 0.63%, and soybean oil 0.22%, plant sterols. They provided about 0.04% and 0.01% plant sterols to the SL or SO diets, respectively. Therefore, it was unlikely that the increased excretion of neutral sterols by rats fed SL diet was mediated through plant sterols. Egg lecithin feeding did not reduce the plasma total cholesterol level, but elevated HDL-C significantly, which, in turn, resulted in a higher HDL-C/TC ratio than lard or soybean oil feeding (Fig. 4.1). Dietary egg lecithin did not increase the fecal excretion of neutral sterols (Fig. 4.2), and thus seemed to have no effect on the intestinal absorption of cholesterol. Dietary EL might affect the cholesterol homeostasis of rats at a post-absorption site, which leads to an elevated reverse transport of cholesterol. The increase of HDL-C by dietary egg lecithin, as compared to dietary soy lecithin, was also observed previously in guinea pigs (O'Brien and Corrigan 1988). The mechanism underlining the elevation of HDL-C in rats or guinea pigs specifically by dietary egg lecithin but not soy lecithin is not clear, indicating a need for further research into this aspect.

Table 4.1. The compositions of experimental diets

Ingredients[1]	g/kg feed
Casein	270
Corn starch	200
Glucose	207.65
Non-nutritive cellulose	50
Vitamin mix	10
Mineral mix	50.85
Choline chloride	2.75
Inositol	6.25
D,L-methionine	2.5
Cholesterol	2.0
Oil[2]	198

[1] Casein, starch, cellulose, inositol, and methionine were purchased from United States Biochemical Co., Cleveland, OH; vitamin mix (Bernhart-Tomarelli), mineral mix (AOAC), and powdered glucose were from Tekland, Madison, WI; choline chloride, cholesterol, and sodium cholate were from Sigma.

[2] The four types of tested oils were: SAFA, a mixture of coconut oil and safflower oil (4/1); YNO, yolk neutral oil; LARD, lard; and PUFA, a mixture of coconut oil and safflower oil (1/4).

Table 4.2. The major fatty acids of the four test oils as determined by the gas chromatography

Fatty acid	SAFA[1]	YNO	LARD	PUFA
	% of total fatty acids			
C8:0	7.4	0	0	3.2
C10:0	5.7	0	0	2.3
C12:0	43.4	0	0	16.6
C14:0	15.5	.6	1.7	5.4
C16:0	7.7	27.1	23.8	7.7
C16:1	0	4.6	3.3	0
C18:0	2.2	6.8	14.5	2.4
C18:1	6.7	48.8	45.3	11.4
C18:2	11.2	10.2	9.6	50.6
C18:3	0	.6	.7	.2
Σ Saturated (S)	82.0	34.8	40.8	37.8
Σ Monounsaturated	6.7	53.4	48.6	11.4
Σ Polyunsaturated (P)	11.3	10.9	10.6	50.8
P/S ratio	.14	.31	.26	1.34

[1] The four types of tested oils were: SAFA, a mixture of coconut oil and safflower oil (4/1); YNO, yolk neutral oil; LARD, lard; and PUFA, a mixture of coconut oil and safflower oil (1/4).

Table 4.3. The feed consumption, body weight gain and feed conversion efficiency of rats on experimental diets during the 28-day test period[1]

Diet[2]	Total feed consumed	Total body weight gain	Feed conversion ratio (g feed/g gain)
	(g)	(g)	
SAFA	557.5 ± 17.8	201.9 ± 10.5	2.8 ± .1
YNO	559.7 ± 15.6	191.3 ± 9.0	2.9 ± .1
LARD	542.4 ± 21.1	188.7 ± 9.0	2.9 ± .1
PUFA	547.2 ± 18.9	195.9 ± 7.5	2.8 ± .1

[1] Mean ± SEM (n = 7).

[2] The four types of tested oils were: SAFA, a mixture of coconut oil and safflower oil (4/1); YNO, yolk neutral oil; LARD, lard; and PUFA, a mixture of coconut oil and safflower oil (1/4).

Table 4.4. The plasma total and high-density lipoprotein (HDL) cholesterol levels of rats at the end of the experiment[1]

Diet[2]	Total cholesterol	HDL-cholesterol	HDL-C/TC ratio
	---------------- mg/ dL	----------------	
SAFA[1]	96.5 ± 3.9[a]	76.4 ± 3.6[a]	.79
YNO	81.1 ± 5.1[b]	64.7 ± 2.2[b]	.79
LARD	76.1 ± 5.6[b]	63.6 ± 4.6[b]	.84
PUFA	69.8 ± 4.5[b]	56.2 ± 4.3[b]	.81

[a,b] Means within a column without common letters differ significantly ($P < .05$).

[1] Mean ± SEM (n = 7).

[2] The four types of tested oils were: SAFA, a mixture of coconut oil and safflower oil (4/1); YNO, yolk neutral oil; LARD, lard; and PUFA, a mixture of coconut oil and safflower oil (1/4).

Table 4.5. Nutrient composition of the experimental diets

Nutrient[2]	Test diets[1]			
	EL	SL	LD	SO
	g/kg			
Casein, high protein	247.2	247.2	247.2	247.2
Glucose, monohydrate	190.5	190.5	190.5	190.5
Corn starch	350.8	350.8	350.8	350.8
DL-methionine	2.4	2.4	2.4	2.4
Choline chloride	1.8	1.8	1.8	1.8
Inositol	5.6	5.6	5.6	5.6
Vitamin Mix, A.O.A.C.	9.3	9.3	9.3	9.3
Mineral Mix, Bernhart-Tomarelli	46.8	46.8	46.8	46.8
Cellulose	20.6	20.6	40.6	40.6
Cholesterol	0	5.0	5.0	5.0
Crude egg lecithin	75.0	0	0	0
Crude soy lecithin	0	70.0	0	0
Lard	50.0	50.0	100.0	50.0
Soybean oil	0	0	0	50.0

[1] The four dietary treatments were: egg lecithin (EL), soy lecithin (SL), lard (LD), and soybean oil (SO).

[2] The following nutrients were purchased from Teklad, Madison, WI, USA: casein, high protein; dextrose, monohydrate; corn starch; DL-methionine; vitamin mix, AOAC; mineral mix, Bernhart-Tomarelli; cellulose; soybean oil and lard. Cholesterol (USP), choline chloride, and inositol were from Sigma Chemical Company, St. Louis, MO, USA. Soy lecithin was purchased from Canada Packer, Ltd., Toronto, Canada.

Table 4.6. The major fatty acids of the experimental diets

Fatty acid	Test diet			
	Egg lecithin (EL)	Soy lecithin (SL)	Lard (LD)	Soybean oil (SO)
	% of total fatty acids			
14:0	0.92	0.81	1.39	0.77
16:0	24.45	19.00	23.51	17.10
16:1	2.02	1.25	2.28	1.28
18:0	15.60	10.68	16.16	10.16
18:1	39.93	30.09	45.18	35.66
18:2n-6	12.18	33.40	10.76	30.93
18:3n-3	0.59	4.78	0.74	4.12
20:4n-6	2.90	0	0	0
P/S[1]	0.38	1.25	0.28	1.25

[1] Ratio of polyunsaturated to saturated fatty acids.

Table 4.7. Initial and final body weights, and daily feed consumption of rats[1]

Diet[2]	Initial body weight	Final body weight	Daily feed consumption
	g	g	g
SL	113 ± 2.8	322 ± 10.3	22.5 ± .7
EL	111 ± 3.2	325 ± 7.1	23.3 ± .6
SO	113 ± 3.9	325 ± 7.8	23.5 ± .8
LD	111 ± 3.5	329 ± 8.8	24.6 ± .8

[1] Mean ± SEM, n = 8.

[2] The four dietary treatments were: egg lecithin (EL), soy lecithin (SL), lard (LD), and soybean oil (SO).

Table 4.8. Tissue size, total lipids, free and total cholesterol contents of rat liver[1]

Diet[2]	Liver	Total lipid	Free cholesterol	Total cholesterol
	(g)	(% of tissue)	(mg/g wet tissue)	
SL	11.1 ± 1.3^{a}	12.3 ± 2.4^{c}	3.6 ± 0.3^{b}	7.9 ± 1.8^{b}
EL	12.7 ± 1.7^{a}	13.0 ± 2.2^{c}	3.5 ± 0.4^{b}	9.3 ± 1.4^{b}
SO	12.8 ± 1.4^{a}	21.0 ± 2.5^{a}	7.2 ± 1.6^{a}	16.1 ± 1.8^{a}
LD	12.9 ± 1.5^{a}	17.4 ± 2.3^{b}	7.3 ± 1.3^{a}	15.6 ± 2.7^{a}

[1] Mean ± SD, n=8. Means in a column without common letters differ significantly ($P < .05$).

[2] The four dietary treatments were: egg lecithin (EL), soy lecithin (SL), lard (LD), and soybean oil (SO).

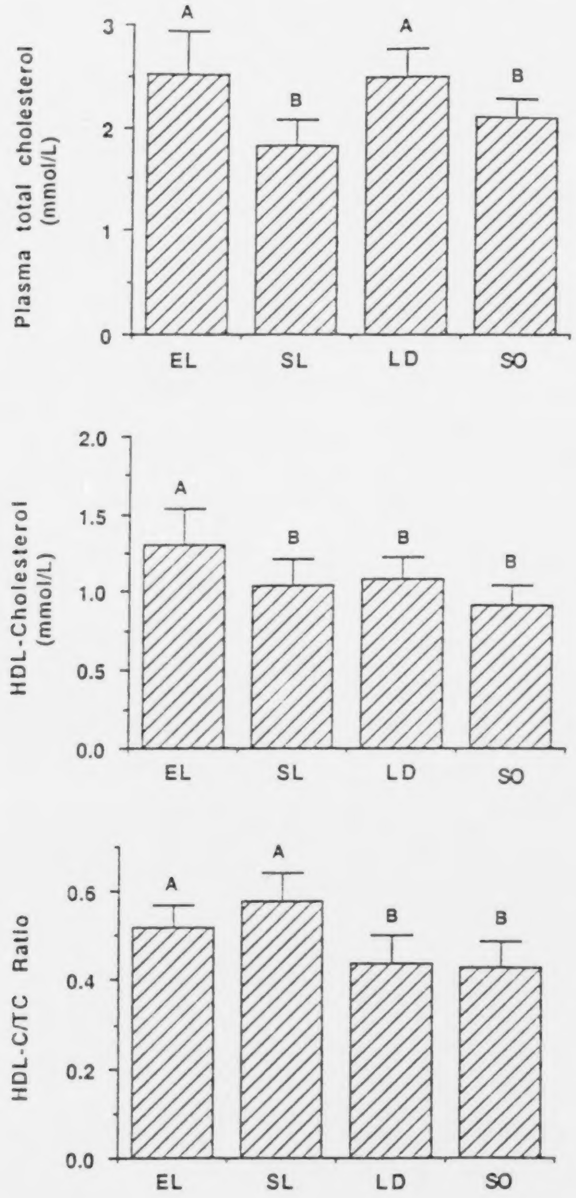

Figure 4.1. Effects of dietary lipids on plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) contents and HDL-C/TC ratios of rats at the end of the feeding trial (Mean ± SD, n=8). The test diets contained egg lecithin (EL), soy lecithin (SL), lard (LD), or soybean oil (SO). Bars in the same graph without common letters differ significantly ($P < .05$).

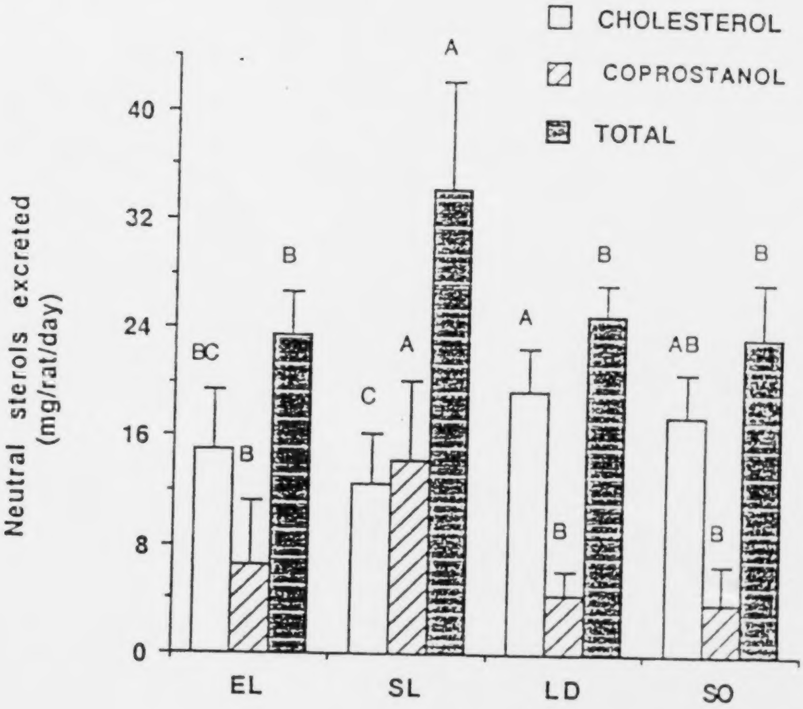

Figure 4.2. Effects of dietary lipids on the fecal excretion of major and total neutral sterols by rats during the fourth wk of the feeding trial (Mean ± SD, n=8). The test diets contained egg lecithin (EL), soy lecithin (SL), lard (LD), or soybean oil (SO). Bars of the same parameter without common letters differ significantly ($P < .05$).

4.3. Reference

Adams, C. W. M. and R. S. Morgan, 1967. The effect of saturated and polyunsaturated lecithins on the resorption of 4-^{14}C-cholesterol from subcutaneous implant. J. Pathol. Bacteriol. 94:73-76.

Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475.

American Heart Association, Nutrition Committee, 1988. Dietary guidelines for healthy American adults. Arteriosclerosis 8:218A-221A.

Baggio, G., A. Pagnan, M. Muraca, S. Martini, A. Oppportuno, A. Bonanome, G. B. Ambrosio, S. Ferrari, P. Guarini, D. Piccolo, E. Manazto, R. Corrocher, and G. Crepaldi, 1988. Olive-oil-enriched diet: effect on serum lipoprotein levels biliary cholesterol saturation. Am. J. Clin. Nutr. 47:960-964.

Bartov, I., R. Reiser, and G. R. Henderson, 1973. Hypercholesterolemic effect in the female rat of egg yolk versus crystalline cholesterol dissolved in lard. J. Nutr. 103:1400-1405.

Becker, N., D. T. Illingworth, P. Alaupovic, W. E. Connor, and E. E. Sundberg, 1983. Effects of saturated, monounsaturated, and omega-6 polyunsaturated fatty acids on plasma lipids, lipoproteins, and apoproteins in human. Am J. Clin. Nutr. 37:355-360.

Byers, S. O. and M. V. Friedman, 1960. Effect of infusions of phosphatides upon the atherosclerotic aorta in situ and as an ocular atherosclerotic implant. J. Lipid Res. 1:342-348.

Burstein, M., H. R. Scholnick, and R. Morfin, 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res. 11:583-595.

Carlson, S. E., and S. Goldfarb, 1977. A sensitive enzymatic method for the determination of free and esterified tissue cholesterol. Clinica Chemica Acta 79:575-582.

Castelli, W. P., R. J. Garrison, P. W. F. Wilson, R. D. Abbott, S. Kalousdian, and W. B. Kannel, 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham study. J. Am. Med. Assoc. 256:2835-2838.

Childs, M. T., J. A. Bowlin, J. T. Ogilvie, W. R. Hazzard, and J. J. Albers, 1981. The contrasting effects of a dietary soya lecithin product and corn oil on lipoprotein lipids in normolipidemic and familial hypercholesterolemic subjects. Atherosclerosis 38:217-228.

Connor, S. L., and W. E. Connor, 1983. The importance of dietary cholesterol in coronary heart disease. Prev. Med. 12:115-123.

Edington, J., M. Geekie, R. Carter, L. Benfield, K. Fisher, M. Ball, and J. Mann, 1987. Effect of dietary cholesterol on plasma cholesterol concentration in subjects following reduced fat, high fibre diet. Brit. Med. J. 294:333-336.

Farquhar, J. W. and M. Sokolow, 1958. Response of serum lipids and lipoproteins of man with beta-sitosterol and safflower oil: a long-term study. Circulation 17:890-899.

Folch, J., M. Lees, and G. H. Slone-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Glueck, C. J., 1979. Dietary fat and atherosclerosis. Am. J. Clin. Nutr. 32:2703-2711.

Greten, H., H. Raetzer, A. Stiehl, and G. Schettler, 1980. The effect of polyunsaturated phosphatidylcholine on plasma lipids and fecal sterol excretion. Atherosclerosis 36:81-88.

Hegsted, D. M., R. B. McGandy, M. L. Myer, and F. J. Stare, 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17:281-295.

Ho, S. Y., and J. Dupont, 1981. Improved enzymatic method for determination of free and esterified cholesterol in plasma lipoproteins and lipid extracts. Fed. Proc. 40:646.

Hollander, D. and D. Morgan, 1980. Effect of plant sterols, fatty acids and lecithin on cholesterol absorption in vivo in the rat. Lipids 15:395-400.

Howard, A. N., J. Patelski, D. E. Bowyer, and G. A. Gresham, 1971. Atherosclerosis induced in hypercholesterolemic baboons by immunological injury, and the effects of intravenous polyunsaturated phosphatidylcholine. Atherosclerosis 14:17-29.

Hunt, C. E. and L. A. Duncan, 1985. Hyperlipoproteinaemia and atherosclerosis in rabbits fed low-level cholesterol and lecithin. Br. J. Exp. Path. 66:35-46.

Jiang, Z., G. Cherian, F. E. Robinson, and J. S. Sim, 1990. Effect of feeding cholesterol to laying hens and chicks on cholesterol metabolism in pre and post-hatch chicks. Poultry Science 69:1694-1701.

Jimenez, M. A., M. L. Scarino, F. Vignolini, and E. Mengheri, 1990. Evidence that polyunsaturated lecithin induces a reduction in plasma cholesterol level and favorable changes in lipoprotein composition in hypercholesterolemic rats. J. Nutr. 120:659-667.

Kannel, W. B. and T. Gordon, 1982. The search for an optimum serum cholesterol. Lancet ii:374-375.

Kesaniemi, Y. A. and S. M. Grundy, 1986. Effects of dietary polyenlphosphatidylcholine on metabolism of cholesterol and triglycerides in hypertriglyceridemic patients. Am. J. Clin. Nutr. 43:98-107.

Keys, A., J. T. Anderson, and F. Grande, 1965. Serum cholesterol responses to changes in the diet. II. The effect of cholesterol in the diet. Metabolism 14:776-787.

Kim, K. N., and I. K. Han, 1985. Effects of different dietary sources of cholesterol, protein and fiber on lipid metabolism in broiler chicks. Korean J. Animal Sci. 27(6):362-366.

Knuiman, J. T., A. C. Beynen, and M. B. Katan, 1989. Lecithin intake and serum cholesterol. Am. J. Clin. Nutr. 49:266-268.

Kuksis, A., 1978. Page 373 in: Handbook of Lipid Research. A. Kuksis, ed. Plenum Press, New York.

Lees, A. M., H. Y. I. Mok, R. S. Lees, M. A. McCluskey, S. M. Grundy, 1977. Plant sterols as cholesterol-lowering agent: clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 28:325-338.

Leotta, S. and M. A. Fusco, 1985. Effects of soya lecithin in a group of diabetic hyperlipidemic patients. Pages 205-209 in: Lecithin and Health Care. Paltauf, F. and D. Lekim, eds., Semmelweise-Institut, Hoya.

Mattson, F. H., and S. M. Grundy, 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. Lipid Res. 26:194-202.

McDonald, P., R. A. Edwards, and J. F. D. Greenhalgh, 1981. In: Animal Nutrition. pp. 189, Longman Inc., New York, NY.

Mensink, R. P., and M. B. Katan, 1989. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high density lipoprotein cholesterol in healthy men and women. N. Engl. J. Med. 321:436-441.

Metcalfe, L. D., A. A. Schmitz, and J. B. Pelka, 1961. The rapid preparation of fatty acid esters for gas chromatography. Anal. Chem. 33:363-364.

Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy, 1965. Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. J. Lipid Res. 6:411-424.

Noble, R. C. 1987. Egg lipids. Pages 159-178 in: Egg Quality-Current Problems and Recent Advances. Wells, R. G. and C. G. Belyvin, eds., Butterworth & Co. Ltd, London.

O'Brien, B. C. and S. M. Corrigan, 1988. Influence of dietary soybean and egg lecithins on lipid responses in cholesterol-fed guinea pigs. Lipids 23:647-650.

O'Mullane, J. E. and J. N. Hawthrone, 1982. A comparison of the effects of feeding linoleic acid-rich lecithin or corn oil on cholesterol absorption and metabolism in the rat. Atherosclerosis 45:81-90.

Oster, K. A., 1982. Letters to the editor. Am. J. Clin. Nutr. 36:1259-1261.

Paul, R., C. S. Ramesha, and J. Ganguly, 1980. On the mechanism of hypocholesterolemic effects of polyunsaturated lipids. Adv. Lipid Res. 17:155-171.

Powrie, W. D., and S. Nakai, 1986. The chemistry of eggs and egg products. Pages 102-105 in: Egg Science and Technology. W. J. Stadalman, and O. J. Cotterill, ed. AVI Publishing Company, Inc., Westport, CT.

Reiser, R., 1973. Saturated fat in the diet and serum cholesterol concentration: a critical examination of the literature. Am. J. Clin. Nutr. 26:524-555.

Reiser, R., J. L. Probstfield, A. Silvers, L. W. Scott, M. L. Shorney, R. D. Wood, B. C. O'Brien, A. M. Gotto, Jr., D. Phil, and W. Insull, Jr., 1985. Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil. Am. J. Clin. Nutr. 42:190-197.

Samochowiec, L., D. Kadlubowska, and L. Rozewicka, 1976. Investigation in experimental atherosclerosis. Part I. The effects of phosphatidylcholine (EPL) on experimental atherosclerosis in white rats. Atherosclerosis 23:305-317.

Sim, J. S. 1991. Extraction and fractionation of oil and lecithin from fresh egg yolk by an aqueous ethanolic solution. Poultry Sci. 70 (Suppl. 1):110.

Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill, New York, NY.

Stehbens, W. E., 1989. The controversial role of dietary cholesterol and hypercholesterolemia in coronary heart disease and atherogenesis. Pathology 21:213-221.

Steinberg, D., 1989. The cholesterol controversy is over. Why it take so long? Circulation 80:1070-1078.

Tall, A. R. and D. M. Small, 1980. Body cholesterol removal: role of plasma high-density lipoprotein. Adv. Lipid Res. 17:1-51.

Tokarska, B., and M. T. Clandinin, 1985. Extraction of egg yolk oil of reduced cholesterol content. Can. Inst. Food Sci. Tech. J. 18(3):256-258.

Warnick, G. R., J. Benderson, and J. J. Albers, 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high density lipoprotein cholesterol. Clin. Chem. 28:1379-1388.

Wong, E. K., R. J. Nicolosi, P. A. Low, J. A. Herd, and K. C. Hayes, 1980. Lecithin influence on hyperlipemia in Rhesus monkeys. Lipids 15:428-433.

Chapter 5. Altering Yolk Fatty Acid Composition:

Changes in Various Yolk Lipid Classes[1]

5.1. Introduction

The previous chapter demonstrated that the type of dietary lipids (saturated versus mono- and polyunsaturated, triglycerides versus phospholipids) plays a major role in determining the cholesterogenic property of the diet. Although dietary cholesterol has a relatively minor effect on plasma cholesterol levels, many attempts have been made to reduce egg cholesterol content but with only little practical success (Naber, 1983; Noble, 1987). An alternative way to reduce the cholesterogenic effects of eggs is by altering of yolk fatty acid composition. The cholesterol-lowering effects of n-6 polyunsaturated fatty acids (PUFA), mainly linoleic acid (LA), have been known for decades, whereas the hypocholesterolemic properties of monounsaturated fatty acid (MUFA), such as oleic acid (OA), and especially the n-3 PUFA, such as linolenic acid (LNA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), have been recognized only recently (Grundy, 1986; Kinsella et al., 1990). The incorporation of these fatty acids into the yolk might thus counteract the cholesterogenic property of the egg.

Diets rich in OA, LA, or LNA result in large increases in their concentrations in yolk total lipids (Cruickshank, 1934; Reiser, 1950;

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[1] A version of this chapter has been published. Jiang, Z., D. U. Ahn, and J. S. Sim, 1991. Poultry Science 70:2467-2475.

Fisher and Leveille, 1957; Wheeler et al., 1959; Murty and Reiser, 1961; Panky and Stadelman, 1969; Navarro et al., 1972; Nwokolo and Sim, 1989; Caston and Leeson, 1990). There is, however, relatively little information available on changes of fatty acids in various yolk lipid classes caused by dietary manipulation (Pankey and Stadelman, 1969). Early studies (Murty and Reiser, 1961; Chen et al., 1965) examined the fatty acid changes in neutral lipids and phospholipids, with no further separation of phospholipids into phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the two major components of yolk phospholipids. It has been reported that the increases in OA or LNA occurred mainly in the triglycerides (Chen et al., 1965), but LA was evenly deposited among triglycerides (TG), lecithin, and cephalin fractions (Pankey and Stadelman, 1969). Studies with human subjects revealed that the n-3 fatty acids of platelet lipids in those who consumed fish or flax oil were not evenly distributed among lipid classes, but were concentrated in the alkenylacyl-PE fraction (Aukema and Holub, 1989; Holub, 1990).

The present study was carried out to examine the effects of laying hen diets enriched with OA, LNA, or LA on the fatty acid compositions of yolk lipid classes. Special attention was given to the distribution of the longer chain n-3 fatty acids in the egg yolk due to their potential health benefits to consumers.

5.2. Materials and Methods

A total of 528 Single Comb White Leghorn laying hens, 16 mo of age, were housed in three double-deck cage batteries with two birds in each cage (.31 x .36 m). Each battery had 88 cages and was divided into four units with 22 cages per unit, thus generating a total of 12 experimental units. The birds were allotted to four dietary treatments with each treatment replicated three times among the batteries in a randomized block design. Four isonitrogenous, isocaloric laying hen diets were prepared (Table 5.1). Full-fat flax seed (Hn-3) and regular high LA sunflower seed (Hn-6) were purchased from local feed producers, and the high OA sunflower seed (Hn-9) was donated (SVO Enterprises, Cleveland, OH 44094). The oil seeds with hull were ground, and incorporated at different levels into laying hen rations to provide similar amounts of dietary fat. Feed and water were provided ad libitum. Egg production was recorded weekly. Egg weight, Haugh units (Haugh, 1937) and specific gravity (Voisey and Hamilton, 1976) were measured at the end of the 4-wk feeding trial using 15 eggs from each experimental unit.

At Day 23 of feeding, two eggs from each of the three 22-cage units, totalling six eggs per treatment, were randomly selected. The eggs were weighed, cracked and yolks were separated. From each yolk sample, about 1.0 g of yolk was weighed, and total yolk lipids were extracted and measured according to Folch et al. (1957). A portion of the lipid extracts was dried under nitrogen, and methylated according to Metcalfe et al. (1961). The remaining portion of the lipid extracts was concentrated under nitrogen to about 150 mg lipids/mL of solvent. Fifty microliter (μL) of the concentrated lipid extracts were applied onto precoated silica gel G plates[3] (20 x 20 cm) that had been previously activated by heating at 120 C for 2 h. Tripalmitine, cholesterol stearate, cholesterol, PC, PE, and phosphatidylserine (PS) (Sigma Chemical Co., St. Louis, MO 63178) were mixed and used as lipid standards. The plates were developed in a chloroform:methanol: water (65:25:4) mixture until the solvent front moved 10 cm from the origin (approximately 25 min). The plates were air- dried and then developed in a hexane:diethyl ether (4:1) mixture until the solvent front moved 17 cm from the origin (approximately 20 min) (Fried and Shapiro, 1979). The plates were then air-dried, and sprayed with .1% (wt/vol) 2', 7'-dichloro-fluorescein in ethanol. Regions corresponding to cholesterol stearate, TG, PC, PE, and PS were identified under ultraviolet light and were scraped into screw-capped tubes for methylation using Boron-trifluoride-methanol (Metcalfe et al., 1961). The fatty acid methyl esters of total lipids and TG, PC, and PE fractions were then separated and quantified by an automated gas chromatograph equipped with an on-column injector (Model 3400, Varian Associates, Inc., Sunnyvale, CA 94089). A DB-23 fused capillary column (30 m by .25 mm inside diameter) (Supelco Canada Ltd., Oakville, Ontario, Canada, L6K 3V1) was used. The initial column temperature was set at 70 C for .3 min, increased to 180 C at 30 C/min and held for 10 min. Then the column temperature was elevated to 230 C at a rate of 5 C/min and held at the final temperature for 3 min. Helium was used as a carrier gas. A flame ionization detector was set at 240 C. A Hewlett-Packard Series 3353 laboratory automation system (Hewlett-Packard, Avondale, PA 19311) was used to integrate peak areas. Quantification of fatty acids in cholesterol esters and PS fractions were not successful due to their low contents in yolk total lipids (1.3 and .8% of total, respectively).

Data pertaining to egg production and egg quality were analyzed using ANOVA for two-way randomized complete block design (diet x battery). A one-way ANOVA was used to analyze the effect of dietary treatments on fatty acid contents of total and various classes of yolk lipids using eggs within treatment as the error term. Treatment means were separated using Student-Newman-Keuls' test ($P<.05$). Simple correlation coefficients between LNA and arachidonic acid (AA), total long chain n-3 fatty acids and AA in various yolk lipid classes were calculated (Steel and Torrie, 1980).

5.3. Results and Discussion

It has been known for more than five decades that yolk fatty acids can be easily altered by dietary manipulation (Cruickshank, 1934). Because dietary lipids play important roles in human health, investigations of the effects of dietary treatment on yolk lipids are extensive (Noble, 1987). Previous studies, however, focused on the effects of dietary fats on the fatty acid composition of yolk total lipids. Thus there is a lack of information about the effects of dietary fats on fatty acid compositions of major classes of yolk lipids (Chen et al., 1965). The present study was carried out to examine the effects of feeding different types of fat on the fatty acid distribution in major yolk lipid classes.

The fatty acid compositions of laying hen diets were greatly changed by the incorporation of flax and sunflower seeds (Table 5.2). Oleic acid, LNA, or LA was the dominant fatty acid in diets containing Hn-9, Hn-3, or Hn-6, respectively. It should be noted that the LNA content of the control diet was relatively high due to the use of a commercial mixture of tallow and vegetable oil to achieve the desired levels of fatty acids for comparison purposes (Table 5.2). Hen-day egg production (74.8%), egg weight (63.5 g), and egg quality in terms of Haugh unit (80.5) and specific gravity (1.075) were not affected by diets. Dietary treatment had no effect on the total lipid content of the egg yolk (33.6%, wt/wt of fresh yolk), which corroborated previous results (Pankey and Stadelman, 1969).

Fatty acid composition of yolk total lipids

The fatty acid composition of yolk total lipids reflected those of the laying hen diets (Table 5.3 and Figure 5.1). The magnitude of change in different fatty acids, however, was different. The ability of laying hens to increase OA in the yolk seemed limited. An increase of 17% ($P<.05$) of OA was found in yolk total lipids, even though the OA content in the Hn-9 diet was twice that of the control diet. On the other hand, LNA and LA contents of egg yolks from Hn-3 and Hn-6 regimes increased by 6.4 and 2.5 times ($P<.05$), respectively, closely resembling their changes in the laying hen diets (6.4 and 2.3 times over the control, respectively).

The lower LNA contents of the two sunflower seed diets were also reflected in the yolk total lipids, resulting in significantly ($P<.05$) lower LNA and total n-3 fatty acid contents in the yolk. Feeding Hn-3 increased LNA content in the yolk by more than six fold over the control ($P<.05$). The longer chain metabolites of LNA, such as EPA, DPA, and DHA, were also increased ($P<.05$), whereas AA, the metabolite of LA, was reduced. Consequently, the ratio of n-6 to n-3 PUFA in yolk total lipids was significantly ($P<.05$) reduced by Hn-3 feeding.

Fatty acid composition of yolk lipid classes

Feeding Hn-9 increased OA content in TG significantly ($P<.05$) with a concomitant reduction of saturated fatty acids ($P<.05$) (Table 5.4). Oleic acid contents in yolk PC and PE, however, were not affected by feeding Hn-9.

The incorporation of LA into yolk lipids caused by feeding Hn-6 reached similar extents in yolk total lipids, TG, and PC, but was much less in PE (Figure 5.1). The metabolite of LA, AA, was increased in TG and PC of yolks from the Hn-6 regime, but not in PE where the highest proportion of AA was found.

The enrichment of LNA upon feeding Hn-3 was mainly in yolk TG with only moderate increase in PC and PE (Figure 5.1). In contrast, EPA, DPA, and DHA, the longer chain metabolites of LNA, were exclusively deposited into yolk phospholipids (Figure 5.1). But even here, these longer chain n-3 fatty acids were not evenly distributed among the major phospholipid classes. Instead, they were concentrated in the yolk PE fraction. The contents of EPA, DPA, and DHA in PE were three to seven times those in PC. Therefore, although PE constituted only 24% and PC 69% of yolk phospholipids (Noble, 1987), PE contained at least an equal amount of the longer chain n-3 fatty acids as PC did. The biochemical mechanisms for this lipid class-specific incorporation of longer chain n-3 fatty acids remain to be elucidated. Because of the plasma source of yolk fatty acids (Christie and Moore, 1972), it would be reasonable to assume that similar changes in fatty acids had also occurred in laying hen plasma lipids. In human subjects, dietary supplement with flax seed or oil resulted in a marked enrichment of platelet alkenylacyl PE, a sub-class of PE, with EPA, DPA, and DHA (Holub, 1990), indicating that this PE-specific incorporation of longer chain n-3 PUFA might not be species-specific for laying hens only.

This study therefore clearly demonstrated that the changes of fatty acids induced by dietary treatment were not uniformly distributed among all lipid classes of egg yolks. Some fatty acids, both endogenous and exogenous, were preferentially deposited by the laying hen in certain yolk lipid classes.

Interactions among fatty acid families

There were interactions among fatty acid families in yolk lipids. The increase of yolk LA or LNA in yolk total lipids and various lipid classes upon feeding Hn-6 or Hn-3 was in place of palmitoleic and oleic acids, which corroborated a previous report (Chen et al., 1965). Feeding Hn-9 and Hn-6 decreased saturated fatty acid content in yolk total lipids and TG, but increased the content in PE ($P<.05$). It is not clear what caused this PE-specific elevation of the saturated fatty acids despite lower dietary levels of saturated fatty acids in Hn-9 and Hn-6 diets.

Another important interaction was between n-3 LNA and n-6 AA. Negative relationships ($P<.05$) between AA and LNA, AA and longer chain n-3 fatty acids were observed in yolk total lipids, TG, PC, and PE. It has been known that the enzymatic pathway for the synthesis of AA from LA is shared by the n-3 fatty acids (Brenner, 1981), and LNA inhibits the $\Delta6$ desaturase and thereby reduces the conversion of LA to AA (Iritani and Narita, 1984; Garg et al., 1988). The higher contents of longer chain n-3 fatty acids, such as EPA, DPA, and DHA, might also hinder the incorporation of AA into PC and PE (Garg et al., 1988; Kinsella et al., 1990) and thus reduced AA content in yolk phospholipids.

Table 5.1. Nutrient composition of laying hen diets

Ingredients and analyses	Laying hen diet[1]			
	Hn-9	Hn-3	Hn-6	Control
	(%)			
Wheat	61.3	66.9	58.7	72.4
Soybean meal	8.0	5.3	7.6	11.8
Flax seed	0	15.0	0	0
Sunflower seed (Hn-9)	18.0	0	0	0
Sunflower seed (Hn-6)	0	0	21.0	0
Fat mixture[2]	0	0	0	3.0
Limestone	8.3	8.3	8.3	8.3
Calcium phosphate	1.9	2.0	1.9	2.0
Salt	.3	.3	.3	.3
DL-methionine	.1	.1	.1	.1
Layer premix[3]	2.1	2.1	2.1	2.1
Calculated analyses:				
CP, %	15.0	15.0	15.0	15.0
ME, kcal/kg	2,732	2,728	2,700	2,751
Ether extracts, %	6.2	6.0	5.9	4.6
Calcium, %	3.6	3.6	3.6	3.6
Available P, %	.5	.5	.5	.5

[1] The laying hen diets contained high oleic acid sunflower seed (Hn-9), full-fat flax seed (Hn-3), and high linoleic acid sunflower (regular seed, Hn-6).

[2] A mixture of animal tallow and vegetable oil from Canada Packers Inc., Toronto, ON, Canada.

[3] Supplied per kilogram of diet the following: vitamin A, 8,000 IU; cholecalciferol, 1,200 ICU; vitamin E, 5 IU; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin B_{12}, 10 μg; choline chloride, 100 mg; biotin, 100 μg; selenium, .1 mg; DL-methionine, 500 mg; manganese sulfate, .4 g; zinc oxide, .1 g.

Table 5.2. Major fatty acids of laying hen diets

Fatty acid	Laying hen diet[1]				SEM
	Hn-9	Hn-3	Hn-6	Control	
	----- (% of total fatty acids[2]) -----				
C16:0	6.9	8.2	9.7	18.0	.24
C18:0	3.8	2.8	4.7	8.5	.11
C16:1(n-7 & n-9)	.5	.6	.8	2.1	.10
C18:1(n-7 & n-9)	65.3	19.4	22.0	35.8	.32
C18:2(n-6)	22.2	23.5	61.1	26.9	.68
C18:3(n-3)	1.2	45.8	1.4	7.2	.17
Σ Saturated	10.7	11.0	14.4	26.5	
Σ MUFA[3]	65.8	20.0	22.8	37.9	
Σ PUFA[4] (n-6)	22.2	23.5	61.1	26.9	
Σ PUFA (n-3)	1.2	45.8	1.4	7.2	
Σ(n-6)/Σ(n-3)	18.5	.5	43.6	3.7	

[1] The laying hen diets contained high oleic acid sunflower seed (Hn-9), full-fat flax seed (Hn-3), and high linoleic acid sunflower (regular seed, Hn-6).

[2] Each value was the mean of three determinations.

[3] MUFA = monounsaturated fatty acids.

[4] PUFA = polyunsaturated fatty acids.

Table 5.3. Effects of dietary fats on fatty acid composition of yolk total lipids

Fatty acid	Laying hen diet[1]				SEM
	Hn-9	Hn-3	Hn-6	Control	
	----- (% of total fatty acids[2]) -----				
C16:0	22.3^b	25.6^a	23.2^b	25.3^a	.34
C18:0	7.7^b	9.6^a	9.9^a	9.4^a	.33
C16:1(n-7 & n-9)	3.1^b	3.1^b	2.3^c	3.9^a	.15
C18:1(n-7 & n-9)	52.3^a	39.0^c	32.1^d	44.7^b	.60
C18:2(n-6)	11.1^b	12.1^b	28.5^a	11.6^b	.68
C18:3(n-3)	$.2^b$	5.8^a	$.3^b$	$.9^b$	.30
C20:4(n-6)	2.2^b	1.3^c	2.7^a	2.0^b	.13
C20:5(n-3)	0^b	$.3^a$	0^b	0^b	.02
C22:5(n-3)	0^b	$.3^a$	$.1^b$	$.2^b$	.05
C22:6(n-3)	$.8^c$	2.7^a	$.5^c$	1.5^b	.12
Σ Saturated	30.2^c	35.5^a	33.4^b	35.1^a	.46
Σ MUFA[3]	55.4^a	42.2^c	34.5^d	48.6^b	.64
Σ PUFA[4] (n-6)	13.4^b	13.4^b	31.2^a	13.6^b	.66
Σ PUFA (n-3)	1.1^c	9.1^a	$.9^c$	2.6^b	.26
Σ(n-6)/Σ(n-3)	12.7^b	1.5^d	34.3^a	5.3^c	1.03

[a-d]:Means in a row with no common superscripts differ significantly (P<.05, n=6).

[1] The laying hen diets contained high oleic acid sunflower seed (Hn-9), full-fat flax seed (Hn-3), and high linoleic acid sunflower (regular seed, Hn-6).

[2] Each value was the mean of six eggs.
[3] MUFA = monounsaturated fatty acids.
[4] PUFA = polyunsaturated fatty acids.

Table 5.4. Effect of dietary fats on fatty acid composition of yolk triglycerides

Fatty acid	Laying hen diet[1] Hn-9	Hn-3	Hn-6	Control	SEM
	----- (% of total fatty acids[2])-----				
C16:0	22.5^{a}	24.5^{a}	22.9^{a}	24.0^{a}	1.02
C18:0	4.3^{b}	6.9^{a}	6.3^{a}	6.8^{a}	.38
C16:1(n-7 & n-9)	3.8^{b}	4.7^{a}	3.1^{c}	4.6^{a}	.09
C18:1(n-7 & n-9)	59.1^{a}	45.8^{c}	39.1^{d}	53.8^{b}	.57
C18:2(n-6)	9.5^{b}	10.7^{b}	27.5^{a}	9.1^{b}	.66
C18:3(n-3)	$.3^{b}$	6.9^{a}	$.5^{b}$	1.1^{b}	.36
C20:4(n-6)	$.1^{b}$	0^{b}	$.3^{a}$	0^{b}	.04
Σ Saturated	27.2^{b}	31.7^{a}	29.5^{ab}	31.2^{a}	1.21
Σ MUFA[3]	62.9^{a}	50.4^{c}	42.1^{d}	58.4^{b}	.54
Σ PUFA[4] (n-6)	9.6^{b}	10.7^{b}	27.8^{a}	9.1^{b}	.67
Σ PUFA (n-3)	$.3^{b}$	7.1^{a}	$.6^{b}$	1.2^{b}	.40
Σ(n-6)/Σ(n-3)	29.9^{b}	1.6^{d}	52.8^{a}	8.1^{c}	1.78

[a-d]: Means in a row with no common superscripts differ significantly ($P<.05$, n=6).

[1] The laying hen diets contained high oleic acid sunflower seed (Hn-9), full-fat flax seed (Hn-3), and high linoleic acid sunflower (regular seed, Hn-6).

[2] Each value was the mean of six eggs.

[3] MUFA = monounsaturated fatty acids.

[4] PUFA = polyunsaturated fatty acids.

Table 5.5. Effect of dietary fats on fatty acid composition of yolk phosphatidylcholine

Fatty acid	Laying hen diet[1]				SEM
	Hn-9	Hn-3	Hn-6	Control	
	----- (% of total fatty acids[2]) -----				
C16:0	30.0^{a}	32.7^{a}	32.9^{a}	32.5^{a}	1.17
C18:0	12.3^{ab}	12.8^{b}	14.8^{a}	11.3^{b}	.48
C16:1(n-7 & n-9)	1.9^{a}	1.8^{a}	1.3^{b}	1.9^{a}	.10
C18:1(n-7 & n-9)	35.8^{a}	31.3^{c}	21.7^{d}	33.7^{b}	.74
C18:2(n-6)	14.8^{b}	13.4^{b}	23.9^{a}	14.2^{b}	1.04
C18:3(n-3)	0^{b}	1.5^{a}	0^{b}	$.2^{b}$	.05
C20:4(n-6)	3.6^{b}	1.7^{d}	4.4^{a}	3.1^{c}	.18
C20:5(n-3)	0^{a}	$.2^{a}$	0^{a}	0^{a}	.03
C22:5(n-3)	0^{b}	$.5^{a}$	0^{b}	$.2^{b}$	.04
C22:6(n-3)	1.5^{c}	4.0^{a}	1.1^{c}	2.8^{b}	.17
Σ Saturated	42.3^{a}	45.6^{a}	47.7^{a}	44.0^{a}	1.51
Σ MUFA[3]	37.7^{a}	33.1^{b}	22.9^{c}	35.6^{a}	.75
Σ PUFA[4] (n-6)	18.4^{b}	15.1^{b}	28.2^{a}	17.3^{b}	1.14
Σ PUFA (n-3)	1.5^{c}	6.1^{a}	1.1^{c}	3.2^{b}	.20
Σ(n-6)/Σ(n-3)	12.2^{b}	2.5^{c}	27.5^{a}	5.5^{c}	1.31

[a-d]: Means in a row with no common superscripts differ significantly (P<.05, n=6).

[1] The laying hen diets contained high oleic acid sunflower seed (Hn-9), full-fat flax seed (Hn-3), and high linoleic acid sunflower (regular seed, Hn-6).

[2] Each value was the mean of six eggs.
[3] MUFA = monounsaturated fatty acids.
[4] PUFA = polyunsaturated fatty acids.

Table 5.6. Effect of dietary fats on fatty acid composition of yolk phosphatidylethanolamine

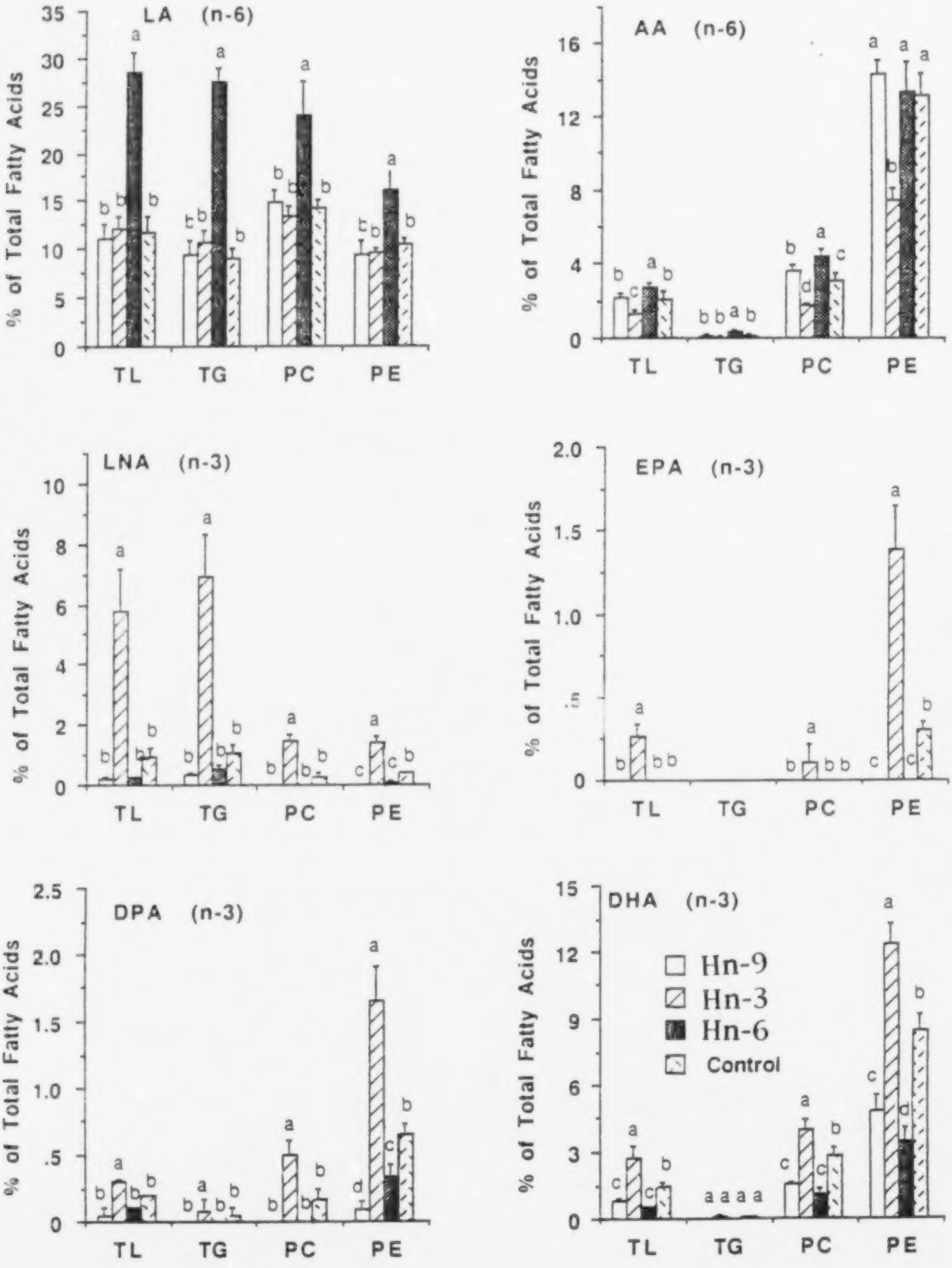
Fatty acid	Laying hen diet[1]				SEM
	Hn-9	Hn-3	Hn-6	Control	
	----- (% of total fatty acids[2]) -----				
C16:0	15.0^{a}	16.4^{a}	15.2^{a}	16.3^{a}	.49
C18:0	34.8^{a}	27.6^{b}	37.8^{a}	27.1^{b}	1.63
C16:1(n-7 & n-9)	$.6^{a}$	$.5^{a}$	$.3^{a}$	$.7^{a}$	.12
C18:1(n-7 & n-9)	21.0^{a}	21.7^{a}	13.3^{b}	22.7^{a}	.98
C18:2(n-6)	9.4^{b}	9.6^{b}	16.1^{a}	10.5^{b}	.63
C18:3(n-3)	0^{c}	1.4^{a}	$.1^{c}$	$.4^{b}$	.04
C20:4(n-6)	14.3^{a}	7.4^{b}	13.3^{a}	13.1^{a}	.52
C20:5(n-3)	0^{c}	1.4^{a}	0^{c}	$.3^{b}$	.07
C22:5(n-3)	$.1^{d}$	1.7^{a}	$.3^{c}$	$.7^{b}$	.08
C22:6(n-3)	4.8^{c}	12.4^{a}	3.5^{d}	8.4^{b}	.38
Σ Saturated	49.8^{a}	43.9^{b}	53.1^{a}	43.3^{b}	2.02
Σ MUFA[3]	21.6^{a}	22.2^{a}	13.6^{b}	23.4^{a}	1.03
Σ PUFA[4] (n-6)	23.7^{b}	17.0^{c}	29.4^{a}	23.6^{b}	1.00
Σ PUFA (n-3)	4.9^{c}	16.8^{a}	3.9^{c}	9.7^{b}	.47
$\Sigma(n-6)/\Sigma(n-3)$	4.9^{b}	1.0^{d}	7.7^{a}	2.4^{c}	.16

$^{a-d}$: Means in a row with no common superscripts differ significantly ($P<.05$, n=6).

[1] The laying hen diets contained high oleic acid sunflower seed (Hn-9), full-fat flax seed (Hn-3), and high linoleic acid sunflower (regular seed, Hn-6).

[2] Each value was the mean of six eggs.
[3] MUFA = monounsaturated fatty acids.
[4] PUFA = polyunsaturated fatty acids.

Figure 5.1. Effects of dietary oil seeds on the distribution of selected fatty acids (x ± SD, n=6) in total and major classes of yolk lipids. Bars in a cluster with no common superscripts differ significantly ($P<.05$). The laying hen diets contained high oleic acid sunflower seed (Hn-9), full-fat flax seed (Hn-3), and high linoleic acid sunflower (regular seed, Hn-6). Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LNA, α-linolenic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triglycerides; TL, total lipids.


LA (n-6)
AA (n-6)
LNA (n-3)
EPA (n-3)
DPA (n-3)
DHA (n-3)
% of Total Fatty Acids
TL
TG
PC
PE
0
5
10
15
20
25
30
35
4
8
12
16
2
6
.5
1.0
1.5
2.0
2.5
3
9
Hn-9
Hn-3
Hn-6
Control
a
b
c
d

5.4. References

Aukema, H. M., and B. J. Holub, 1989. Effect of dietary supplementation with a fish oil concentrate on the alkenylacyl class of ethanolamine phospholipid in human platelets. J. Lipid Res. 30:59-64.

Brenner, R. R., 1981. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog. Lipid Res. 20:41-47.

Caston, L., and S. Leeson, 1990. Dietary flax and egg composition. Poultry Sci. 69:1617-1620.

Chen, P. H., R. H. Common, N. Nikolaiczuk, and H. F. MacRae, 1965. Some effects of added dietary fats on the lipid composition of hen's egg yolk. J. Food Sci. 30:838-845.

Christie, W. W., and J. H. Moore, 1972. The lipid components of the plasma, liver and ovarian follicles in the domestic chicken (Gallus gallus). Comp. Biochem. and Physiol. 41B:287-295.

Cruickshank, E. M., 1934. Studies in fat metabolism in the fowl. 1. The composition of the egg fat and depot fat of the fowl as affected by the ingestion of large amounts of different fats. Biochem. J. 28:965-977.

Fisher, H., and G. A. Leveille, 1957. Observations on the cholesterol, linoleic and linolenic acid content of eggs as influenced by dietary fats. J. Nutr. 63:119-129.

Folch, J., M. Lees, and G. H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Fried, B., and I. L. Shapiro, 1979. Thin-layer chromatographic analysis of phospholipids in Echinostoma revolutum (trematoda) adults. 65:243-245.

Garg, M. L., E. Sebokova, A. B. R. Thomason, and M. T. Clandinin, 1988. Δ-6 desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ω3 fatty acids. Biochem. J. 249:351-356.

Grundy, S. M., 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N. Engl. J. Med. 314:745-748.

Haugh, R. R., 1937. The Haugh unit for measuring egg quality. U.S. Egg Poult. Mag. 43:552-555, 572-573.

Holub, B. J., 1990. Effects of flax and dietary alpha-linolenic acid on blood platelets in human subjects. Pages 12-15 in: Proceedings of the 53rd Flax Institute of the United States. Fargo, ND.

Iritani, N., and R. Narita, 1984. Changes of arachidonic acid and n-3 polyunsaturated fatty acids of phospholipid classes in liver, plasma and platelets during dietary fat manipulation. Biochim. Biophys. Acta 793:441-448.

Kinsella, J. E., B. Lokesh, and R. A. Stone, 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52:1-28.

Metcalfe, L. D., A. A. Schmitz, and J. B. Pelka, 1961. The rapid preparation of fatty acid esters for gas chromatography. Anal. Chem. 33:363-364.

Murty, N. L., and R. Reiser, 1961. Influence of graded levels of dietary linoleic and linolenic acids on the fatty acid composition of hens' eggs. J. Nutr. 75:287-294.

Naber, E. C., 1983. Nutrient and drug effects on cholesterol metabolism in the laying hen. Fed. Proc. 42:2486-2493.

Navarro, J. G., J. C. Saavedra, F. B. Borie, and M. M. Caiozzi, 1972. Influence of dietary fish meal on egg fatty acid composition. J. Sci. Food Agric. 23:1287-1292.

Noble, R. C., 1987. Egg lipids. Pages 159-177 in: Egg Quality -- Current Problems and Recent Advances. R. G. Wells and C. G. Belyavin, ed., Butterworths & Co. Ltd, London, England.

Nwokolo, E., and J. S. Sim, 1989. ω-3 fatty acid enrichment of broiler and layer tissues, and egg yolk by feeding flax and canola seed diets. Poultry Sci. 68(Suppl. 1):106 (Abstr).

Pankey, R. D., and W. J. Stadelman, 1969. Effect of dietary fats on some chemical and functional properties of eggs. J. Food Sci. 34:312-317.

Reiser, R., 1950. Fatty acid changes in egg yolk of hens on fat-free and a cottonseed oil ration. J. Nutr. 40:429-440.

Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., New York, NY.

Voisey, P. W., and R. M. G. Hamilton, 1976. Notes on the measurement of egg specific gravity to estimate egg shell quality. Report 7322-598. Engineering Research Service, Agriculture Canada, Ottawa, ON, Canada.

Wheeler, P., D. W. Peterson, and G. D. Michaels, 1959. Fatty acid distribution in egg yolk as influenced by type and level of dietary fat. J. Nutr. 69:253-260.

Chapter 6. Effects of Yolk Fatty Acid Modification on Cholesterol and Fatty Acid Metabolism in Rats[1]

6.1. Introduction

The previous chapter demonstrated that egg yolks can be enriched with n-6 and n-3 polyunsaturated fatty acids (PUFA) by altering the type of dietary fat in the laying hen rations. The past decade has witnessed a burst of interest in dietary n-3 polyunsaturated fatty acids (n-3 PUFA) and a growing consensus that consumption of n-3 PUFA reduces the risk of coronary heart disease (Bang et al., 1980; Kinsella et al., 1990). Alpha-linolenic acid (LNA), the major plant n-3 fatty acid, exhibited similar beneficial effects to the longer chain n-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that occur in fish oil (Singer et al., 1986; Garg et al., 1988, 1989). The major mechanisms of the ameliorative and preventive effects of n-3 PUFA against atherosclerosis include: 1) inhibition of synthesis of eicosanoids from arachidonic acid which are vasoconstricting and platelet aggregating; 2) reduction of very-low density lipoprotein synthesis, thus reducing plasma triglycerides and cholesterol (Kinsella et al., 1990). It was thus suggested that 800 to 1100 mg per day of LNA and 300 to 400 mg per day of a combination of EPA and DHA are needed to prevent n-3 fatty acid deficiency in the elderly (Simopoulos, 1989). Recently, the Canadian government adopted a recommendation that both n-6 and n-3 PUFA are essential nutrients and that the dietary supply should be at least 3 and 0.5%, respectively, of energy intake (Health and Welfare Canada, 1990). The absolute amount of dietary n-3 fatty acids should be increased, and the ratio of n-6 to n-3 PUFA be reduced (Budowski and Crawford, 1986; Health and Welfare Canada, 1990).

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[1] A version of this chapter has been accepted for publication. Jiang, Z., and J. S. Sim, 1992. Lipids (in press).

An alternative way to provide a dietary supply of n-3 PUFA is by enriching the lipid components of animal products with n-3 PUFA through dietary manipulation. The chicken egg appears to be quite suitable for this purpose. As demonstrated in the previous chapter, nutritionally important n-3 PUFA were incorporated into yolk lipids by feeding laying hens diets containing α-linolenic acid (LNA). Significant amount of longer chain n-3 PUFA were also incorporated into yolk phospholipid fraction, indicating an active conversion of LNA to its longer chain metabolites by the laying hen. The incorporation of n-3 PUFA into yolk lipids and the consequential reduction of n-6/n-3 PUFA ratio might be of special importance in terms of altering the cholesterolemic action of egg yolks. This chapter presents the results of two experiments which examine the effects of yolk fatty acid modification on cholesterol and fatty acid metabolism in rats. In the first experiment, the cholesterolemic properties of regular yolks or yolks enriched with either n-6 or n-3 PUFA were compared. The second study further investigated the influences of fatty acid modification on cholesterolemic actions, rat hepatic activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), a rate-limiting enzyme in cholesterogenesis, and the rate of prostaglandin E_2 synthesis by rat epitrochlearis muscle.

6.2. Materials and Methods

6.2.1. Experiment 1

Laying hen diets and yolk powder preparation

Single Comb White Leghorn pullets, 22 wk of age, were transferred to the test diets (Table 6.1), and the lighting was increased from 8 to 13 hr per day for the first wk, to 14 hr per day during the second wk and maintained at 14 hr per day thereafter. Test diets, containing 10% full-fat flax seed, 12% sunflower seed, or soybean meal plus animal tallow, were provided ad libitum. The fatty acid composition of eggs was monitored, and the changes in fatty acid composition of the yolks were found to reach a plateau two weeks after feeding the test diets. Ten dozen eggs laid during the sixth week were collected from each treatment. The eggs were hard-boiled, and the yolks were separated, pulverized and dried at room temperature. Yolk powders thus obtained contained 96.3% dry matter. The fatty acid compositions of the yolk powder preparations were determined by gas chromatography (Table 6.2).

Animals and diets

Twenty-one female Sprague-Dawley rats, 4 wk of age, were housed individually in metabolic wire cages in a room with controlled temperature (21 C), humidity (60%) and lighting (12 hr light-dark cycle). Water and feed were provided ad libitum. After feeding a commercial diet (Wayne Rodent Blox, Continental Grain Company, Chicago, IL) for three days, they were divided into three groups with seven rats each, in such a manner that the mean body weight of each group was similar.

Three test diets were prepared by mixing 85% basal mix with 15% egg yolk powder (Table 6.3). These test diets contained yolk powders prepared from eggs laid by laying hens fed diets containing 10% flax seed (Hn-3), 12% sunflower seed (Hn-6), and soybean meal plus animal tallow (CON). The diets were stored at 4 C in dark plastic bags. Fresh feed was provided every two days, and feed consumption and body weight gain were measured weekly.

Sample preparation and analyses

At the end of the 28-day feeding trial, rats were fasted overnight and sacrificed by decapitation the following morning (09:00 to 11:00 h). Trunk blood was collected into Na^+-heparin coated tubes. Rat liver tissue was quickly excised, blotted dry, weighed, and stored at -20 C for lipid analyses. Plasma was separated by centrifugation at 2,000 x g for 30 min. Plasma total cholesterol (TC) content was measured by an enzymatic method (Sigma Diagnostics, Procedure No. 352, St. Louis, MO).

A portion (1.0 g) of the median lobe of rat liver was homogenized with a mixture of $CHCl_3$ and CH_3OH (2:1, vol/vol) (Folch et al., 1957) with 2 mg 5-α-cholestane added as an internal standard, and the liver total lipid content was determined gravimetrically. Liver cholesterol was determined by gas chromatography as described previously (Fenton and Sim, 1991). Aliquots of the liver lipid extracts were dried under nitrogen and converted to their methyl esters by incubation with a mixture of BF_3-methanol (10% BF_3 in methanol), hexane and methanol (35:20:45, vol/vol/vol) at 90 C for 30 min (Metcalfe et al., 1961). Liver phospholipids were separated on the silica gel G plates (Sigma Chemical Co., St. Louis, MO) after developing the plates in a solvent mixture of hexane, diethyl ether and acetic acid (80:20:1) for 30 min. Regions corresponding to the phospholipid standards was scraped into screw-capped tubes and methylated directly in the same way as for the liver total lipids. The methyl esters of fatty acids of liver total lipids and phospholipids were separated and quantified by gas chromatograph equipped with an on-column injector (Varian 3400, Varian Associates Inc., Sunnyvale, CA) using an DB-23 fused capillary column (30 m by .25 mm ID) (J & W Scientific, Folsom, CA). A two-stage column temperature program was used. The initial temperature was set at 70 C for .3 min, increased to 180 C at 30 C/min and held for 10 min. Then the column temperature was elevated to 230 C at a rate of 5 C/min and held at the final temperature for 3 min. Helium was used as the carrier gas. The flame ionization detector was set at 240 C.

6.2.2. Experiment 2

Eggs were collected from laying hens fed diets containing 15% full-fat flax seeds (Hn-3), 21% regular high linoleic acid sunflower seeds (Hn-6), or 18% high oleic acid sunflower seeds (Hn-9) as described in Chapter 5 of this thesis (see Table 5.1 for the laying hen diet). The fatty acid composition of yolk total lipids was determined as described in Chapter 5 and presented in Table 5.3. Egg yolk powders were prepared as described in Experiment 1. Three rat test diets were formulated to contain 15% dry yolk powders (Table 6.3).

Thirty female Sprague-Dawley rats, 4 wk of age, were taken care of as described in Experiment 1, with the only difference being in the light-dark cycle (light 15:00 to 03:00 and dark 03:00 to 15:00). They were fed a commercial diet (Wayne Rodent Blox, Continental Grain Company, Chicago, IL) for 1 wk before being placed on experimental diets. The feeding trial lasted 31 days. At the end, rats were fasted for 12 hr, and in the following morning (08:00 to 10:00, mid-dark period for rats), rats were anaesthetized with halothane (Laboratoires Ayerst, Montreal, Canada). Blood was collected into Vacutainers (Becton Dickinson Canada Inc., Mississague, Ontario, Canada) by heart puncture. Rat liver tissues were quickly excised and a portion of the tissue (about 1 gram) was placed into 10 mL of ice-chilled .9% NaCl solution for the preparation of liver microsomes. Rat epitrochlearis muscles were immediately disected, weighed, and placed into 3 mL of Kreb Ringer's bicarbonate buffer at 37 C for determination of prostaglandin E_2 synthesis.

The HDL_2 fraction was prepared under the optimal conditions for rat plasma (Sjoblom and Eklund, 1989). Plasma TC, HDL_2 cholesterol, and liver total cholesterol and lipid contents were determined as described in Experiment 1. Various fractions of rat liver lipids were separated by thin-layer chromatography as described in Chapter 5 of this thesis and the fatty acid composition of these fractions were determined by gas chromatography.

Rat hepatic microsomes were prepared essentially according to Brown et al. (1979). The microsomal 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) activity was assayed according to Garg and Sabine (1988). In brief, about 100 μg of microsome protein was pre-incubated for 5 min at 37 C in 50 μL of .1 M phosphate buffer (pH 7.4) containing 10 mM EDTA and 50 mM dithioerythritol (DTT). Then 30 μL of .02 M-EDTA/KOH, pH 6.8, containing 5 μmol of glucose-6-phosphate, .5 μmol NADP$^+$, and 1 unit of glucose-6-phosphate dehydrogenase were added. After another 5 min, 30 μL of .1 M phosphate buffer containing 90 nmol of 3 hydroxy-[3-^{14}C]-3-methylglutaryl-CoA (2500 dpm/nmol, New England Nuclear) were added to initiate the assay. After incubation for 30 min in a shaking water bath, the reaction was stopped with 25 μL of 4M HCl containing 4 μmol of [5-^{3}H]-mevalonic acid (500 dpm/μmol, New England Nuclear). The incubation medium was then lactonized by incubating at 37 C for at least 15 min, and centrifuged to sediment proteins. One hundred μL of the supernatant were applied onto the pre-activated silica gel G plates (Sigma Chemical Co., St. Louis, MO). After developing the plates in benzene/acetone (1:1), the region (Rf .5 - .8) containing mevalonolactone was scraped directly into scintillation vials and counted for ^{14}C and ^{3}H radioactivity. The microsome protein content was assayed according to Hartree (1972) using bovine serum albumin as standards. Enzyme activity was expressed as pmol of mevalonic acid synthesized per min per mg of microsome protein.

To study the effects of consuming omega-3 enriched eggs on prostaglandin synthesis, rat epitrochlearis muscle was dissected right after the rat was killed. The muscle was weighed, and pre-incubated for 30 min at 37 C in 3 mL of Kreb-Ringer Bicarbonate (KRB) buffer containing 5 mM glucose and .1 IU/mL insulin (Olomu and Baracos, 1991). The muscle was then transferred to 3 mL of the fresh KRB buffer and incubated for another 2 hr. Throughout the pre- and the assay incubation, the medium was continuously gassed with a mixture of oxygen and carbon dioxide (19/1, vol/vol) and temperature was maintained at 37 C in a shaking water bath.

The incubation was stopped by removing the muscle. The amount of prostaglandin E_2 released by the muscle into incubate medium was determined by radioimmunoassay as described by Olomu and Baracos (1991). The rate of synthesis was expressed as pg of PG E_2 released into medium by mg of musle per hr.

6.2.3. Statistical Analyses

The effects of diets were analyzed by Analysis of Variance followed by Student-Newman-Keuls' test in both experiments (SAS Institute, 1985).

6.3. Results

Fatty acid profiles of yolk lipids

The fatty acid composition of the yolk powders were readily modified by the laying hen test diets (Table 5.3 and 6.2). LNA was the major n-3 PUFA deposited in Hn-3 yolks, although considerable amounts of EPA and DHA were also found in these yolks. Feeding flax seed to laying hens also slightly increased yolk LA and decreased yolk AA content. On the other hand, yolk powder from laying hens fed high linoleic acid sunflower seed diet more than doubled the n-6 linoleic acid (LA) in place of oleic acid. Consequently, the ratios of LA/LNA and Σn-6/Σn-3 PUFA were dramatically decreased in Hn-3 yolk powders, and increased in Hn-6 yolk powder preparation, when compared to the CON or Hn-9 diets. As a result of higher content full-fat flax seeds in the laying hen diet in Experiment 2 than that in Experiment 1, higher n-3 PUFA levels were also found in the Hn-3 yolks used in Experiment 2 than those used in Experiment 1 (Table 5.3 and 6.2).

Rat performance and organ weights

In both experiments, the test diets did not affect the daily feed consumption or body weight gain, nor the absolute weight or the percentage of body weight of liver, and heart tissues (Table 6.4 and 6.5), indicating that enrichment of yolks with either n-3 or n-6 PUFA did not adversely affect the overall feeding values of the yolk.

Plasma and liver lipid levels

Feeding the Hn-3 diets significantly reduced plasma total cholesterol (TC) levels in both experiments when compared to the CON or Hn-9 diets (Figure 6.1 and 6.2). The HDL_2 cholesterol levels were not affected by dietary treatment in Experiment 2 (Figure 6.2). Rats fed the Hn-3 diets had also the lowest liver cholesterol contents in both experiments. The Hn-6 diet reduced plasma TC level to the same extent as the Hn-3 diet did in Experiment 1 but was not as effective as the Hn-3 diet in lowering plasma TC in Experiment 2. In both experiments, however, the Hn-6 diets elevated the hepatic cholesterol concentration significantly. As the weight of rat livers was not affected by dietary treatments in either experiment (Table 6.4 and 6.5), the Hn-3 diets thus significantly reduced hepatic cholesterol pool while Hn-6 augmented this pool. Liver total lipid content was also highest in rats fed the Hn-6 diet in Experiment 1 (Figure 6.1).

HMGR activity and the rate of PG E_2 synthesis

No statistically significant changes were found in the HMGR activity of rat hepatic microsomes in Experiment 2 (Table 6.6). The rate of PG E_2 synthesis by rat epitrochlearis muscle, however, was influenced by dietary treatment. Rats fed the Hn-3 diet had a significantly lower ($P<.05$) PG E_2 synthesis rate than those fed the Hn-6 diet (Table 6.6).

Fatty acids of liver total and phospholipids in Experiment 1

The fatty acid composition of liver total lipids was changed by rat test diets (Table 6.7). Significantly higher contents of LNA and its metabolites EPA, DPA, and DHA, were found in liver total lipids of rats fed Hn-3 than those fed CON. In rats fed the n-6 PUFA-enriched yolk powder diet, neither total nor individual n-3 PUFA was changed when compared to the control fed. The Hn-6 diet, although it significantly increased LA content, had no effect on the AA level of liver total lipids.

The LNA content of the liver phospholipid fraction was not affected by dietary treatments (Table 6.8). EPA in liver phospholipids of rats fed Hn-3 diet was .9% of total fatty acids, a significant increase over those in Hn-6 or CON fed rats. Hn-3 feeding compared to CON feeding also increased DHA and reduced AA contents significantly in liver phospholipid fractions. The Hn-6 diet increased LA content of liver phospholipids, but did not elevate AA content.

Fatty acid composition of plasma lipids of rats in Experiment 2

The concentrations of LNA, EPA, and DHA in rat plasma total lipids were all significantly elevated while that of AA was reduced by Hn-3 feeding (Table 6.9). The n-6/n-3 PUFA ratio was consequently decreased in rats fed the Hn-3 diet. Feeding the Hn-6 diet significantly increased the

LA content in rat plasma.

Fatty acid composition of liver phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of rats in Experiment 2

Feeding the Hn-3 diet did not increase the LNA content in either PC or PE fractions of rat liver tissue (Table 6.10 and 6.11), but significantly elevated EPA and DHA concentrations in PC and particularly in PE. The reduction of AA level by the Hn-3 diet was also significant in both PC and PE fractions. The Hn-6 diet did not result in an increase of either LA or AA content in PC or PE fraction of rat liver tissue when compared to the Hn-9.

6.4. Discussion

Since n-3 fatty acids play important roles in human health, particularly in cardiovascular disease, major nutritional advisory groups have recommended to increase the intake of n-3 fatty acids and to lower dietary n-6 to n-3 fatty acid ratio (Budowski and Crawford, 1986; Health and Welfare Canada, 1990). In the present study, chicken eggs were employed to provide the much needed n-3 PUFA and to lower the n-6 to n-3 ratio. A large egg from laying hens fed the 15% flax diet, for example, provided about 600 mg of n-3 PUFA, more than 100 mg of which were longer chain n-3 PUFA such as EPA, DPA, and DHA. Furthermore, the n-6 to n-3 ratio was reduced from 15 or 20 in commercial produced eggs (Sim et al., 1991a; Simopoulos, 1991) to 1.4 in our n-3 PUFA-enriched eggs.

The intake of the n-3 PUFA-enriched eggs reduced not only plasma total cholesterol, but also liver cholesterol. Activity of the rate-limiting enzyme in cholesterol synthesis, HMGR, was not significantly affected by the dietary treatment, indicating that the reduction of body cholesterol pool by the Hn-3 diet was not due to inhibition of de novo synthesis of cholesterol by the liver tissue. A recent study with fish oil also demonstrated that activity of HMGR of rat hepatic microsomes was not affected by 5% dietary fish oil although plasma cholesterol was reduced significantly (Al-Shurbaji et al., 1991). The HMGR activity reported in the current study was lower than those reported by Brown et al. (1979). This might be due to a general suppression of HMGR activity in the liver due to the presence of about .36% cholesterol in rat test diets.

In contrast to the effects of the Hn-3 diet, the Hn-6 diet resulted in the accumulation of more cholesterol in rat liver tissue while reducing the plasma cholesterol content. Thus, eggs enriched with n-6 PUFA were not as potent as those enriched with n-3 PUFA in reducing the body cholesterol pool. This discrepancy was attributable to the different effects of dietary n-3 and n-6 PUFA on cholesterol metabolism in rats. The accumulation of cholesterol in hepatic tissue, often referred to as the redistribution of cholesterol, was previously observed in rats fed vegetable oils rich in LA (Bloomfield, 1964; Kellogg, 1974; Garg et al., 1988).

The increase of n-3 or n-6 PUFA in yolks was in place of monounsaturated fatty acids (MUFA, Table 5.3 and 6.2). The hypocholesterolemic effect of dietary MUFA was reported in human subjects (Grundy, 1986). In the present study, however, replacement of MUFA by n-3 and n-6 PUFA resulted in a reduction of plasma cholesterol levels, indicating that dietary n-3 or n-6 PUFA were more potent than dietary MUFA in lowering plasma cholesterol in rats. Previous studies have also revealed that substitution of MUFA for n-6 PUFA resulted in higher plasma and liver cholesterol levels in rats (Sugano et al., 1983; Kris-Etherton et al., 1984; Watanabe et al., 1984; Clifford et al., 1986).

When dietary n-3 PUFA were provided exclusively as LNA from vegetable oils such as flax and canola oils, a concentration higher than 12% of total fatty acids was needed to effect a lower plasma cholesterol in rats (Garg et al., 1988, 1989; Sim et al., 1991b). In the present study, Hn-3 diet contained 8 to 10% total n-3 PUFA but still lowered plasma and liver cholesterol significantly. The n-3 PUFA-enriched yolks contained more than 3% EPA, DPA, and DHA in addition to about 5.5% LNA, while flax, soybean and canola oils contained LNA as the only n-3 PUFA. The longer chain n-3 PUFA (EPA and DHA) might be more potent than LNA in lowering plasma cholesterol (Nestel, 1986). In addition, the major portion of the longer chain n-3 PUFA (EPA, DPA, and DHA) in the n-3 yolks resided in phospholipid classes, particularly in phosphatidylethanolamine (Chapter 5 of this thesis). As demonstrated in Chapter 4 of this thesis and in many other studies (O'Brien and Corrigan, 1988; Jimenez et al., 1990), dietary phospholipids exhibits a specific and systemic influence on cholesterol metabolism, and the degree of the unsaturation of dietary phospholipids directly influenced rat plasma cholesterol level. The enrichment of yolk phospholipids with EPA, DPA, and DHA might thus enhance the ability of lipid modification by n-3 PUFA-enriched yolks. Furthermore, female rats were used in these studies. The plasma cholesterol of female rats was more responsive to dietary manipulation than that of male rats (Bartov et al., 1973; Terpstra et al., 1982) which were used in the studies of Garg et al.

(1988, 1989) and Sim et al. (1991b).

The consumption of the n-3 PUFA-enriched yolks not only diminished the body cholesterol pool, but more importantly enriched plasma and tissue total and various fractions of phospholipids with LNA, EPA, DPA, and DHA. In addition, the AA content of liver phospholipids, particularly in PE, was significantly reduced. The reduction of AA content could be attributable to the inhibition of AA synthesis by the n-3 PUFA in the Hn-3 diet and/or a lower dietary AA content of Hn-3 yolks.

The incorporation of longer chain n-3 PUFA into tissue phospholipids reduced the ratio of AA to EPA plus DHA. Upon appropriate stimulation, AA and EPA of tissue phospholipids, particularly those of PE fraction, might serve as the immediate precursor molecules for 2- and 3-series eicosanoids, respectively (Aukeman and Holub, 1989). Thus, both the enrichment with n-3 PUFA and the reduction of AA content in tissue phospholipids might affect the synthesis of eicosanoids by animal tissues. Indeed, the synthesis of PG E_2 by the epitrochlearis muscle was reduced in rats fed the Hn-3 diet than in those fed the Hn-6 diet. In a recent study with chicks, Olomu and Baracos (1991) reported that inclusion of flax seed oil in the diet reduced the rate PG E_2 synthesized by skeletal muscles in a dose-dependent manner. The changes in the quality and quantity of eicosanoids synthesized caused by incorporation of n-3 PUFA into tissue lipids is believed to be one of the major mechanisms by which the dietary n-3 PUFA exhibit their ameliorative and preventive effects against atherosclerosis (Kinsella et al., 1990).

When rats were fed the n-6 PUFA-enriched yolk powders, the LA and AA content of plasma total lipids increased. However, a consistent observation was that the AA contents of liver total and phospholipids were not increased by the Hn-6 feeding. In general, increasing dietary intake of LA tends to elevate tissue AA content. However, often no change or even a decrease of tissue AA content was observed when diets were rich in LA (Mahfouz et al., 1984; Garg et al., 1988). This has been attributed to an inhibition of tissue Δ6 desaturase activity by high dietary level of LA (Brenner, 1981; Mahfouz et al., 1984).

In conclusion, the cholesterolemic and lipid modulating properties of dietary egg yolks could be modified by altering the fatty acid composition of yolk lipids through nutritional manipulation of laying hens. The n-3 PUFA-enriched animal products (eggs and meats) could become an important dietary source of nutritionally important n-3 PUFA. Cautions, however, must be exercised in extrapolating the rat data to man, and this warrants further studies with human subjects with the n-3 PUFA-enriched eggs.

Table 6.1. Compositions of laying hen diets for Experiment 1

Ingredients	Laying hen test diets		
	10% Flax	12% Sunflower seed	Control
		%	
Wheat	64.66	62.21	68.69
Soybean meal	10.28	12.01	14.38
Flax seed	10.00	0	0
Sunflower seed	0	12.00	0
Animal tallow	3.25	2.00	5.08
Limestone	8.33	8.34	8.35
Dicalcium phosphate	1.00	0.98	1.01
Salt	0.29	0.29	0.28
DL-methionine	0.09	0.07	0.09
Layer premix[1]	2.10	2.10	2.10
Calculated analyses			
Crude protein (%)	16.5	16.5	16.5
ME (Kcal/kg)	2700	2747	2700

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[1] Supplied per kilogram diet the following: Vitamin A, 8,000 IU; vitamin D_3, 1,200 ICU; vitamin E, 5 IU; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin B_{12}, 10 μg; choline chloride, 100 mg; biotin, 100 μg; DL-methionine, 500 mg; selenium, 100 μg; manganese, 146 mg; zinc, 58 mg.

Table 6.2. Major fatty acids of yolk powders used in Experiment 1

	Yolk powders[1]		
	Hn-3	Hn-6	CON
	% of total fatty acids		
16:0	23.7	25.2	23.0
16:1	3.4	2.8	3.2
18:0	8.5	9.0	9.1
18:1	43.8	36.6	51.4
18:2(n-6)	10.6	21.8	8.8
18:3(n-3)	5.5	0.5	0.2
20:4(n-6)	0.8	2.2	1.8
20:5(n-3)	0.2	0	0
22:6(n-3)	1.9	0.6	0.8
Σn-3	7.9	1.1	1.3
Σn-6	11.4	24.3	10.7
18:2(n-6) /18:3(n-3)	1.9	43.6	44.0
Σn-6/Σn-3	1.4	22.1	8.2

[1] Yolk powders were prepared from eggs of laying hens fed diets containing 10% full-fat flax (Hn-3), 12% sunflower seed (Hn-6), or soybean meal plus animal tallow (CON).

Table 6.3. Composition and proximate analyses of rat test diets used in Experiment 1 and 2

Ingredient[1]	Experiment 1	Experiment 2
	g/kg feed	
Casein, 87.3% CP	175.0	175.0
Yolk powder[2], 34.5% CP	150.0	150.0
Corn starch	360.0	367.4
Glucose, monohydrate	200	200.0
DL-methionine	2.6	2.6
Choline chloride	1.8	0
Inositol	5.6	0
Vitamin mix, A.O.A.C.	10.0	10.0
Mineral mix, Bernhart-Tomarelli	45.0	45.0
Cellulose	50.0	50.0
Proximate analyses of the test diets[2]		
Protein (%)	20.1	20.0
Total lipid (%)	9.9	9.5
Cholesterol (%)	.37	.37

[1] All nutrients, except yolk powder, were purchased from Teklad, Madison, WI.

[2] The mean of three diets with three determinations for each diet.

Table 6.4. Rat performance and the size of liver and heart tissues of rats in Experiment 1

	Diet[1]		
	Hn-3	Hn-6	CON
Initial body wt (g)	80.3 ± 1.9	82.7 ± 2.7	82.7 ± 1.9
Final body wt (g)	208.8 ± 6.6	210.6 ± 5.1	207.2 ± 5.8
Feed consumption (g/rat/day)	16.5 ± .5	16.6 ± .4	16.7 ± .4
Liver tissue (g)	7.1 ± .3	7.7 ± .2	7.2 ± .3
Heart tissue (g)	.9 ± 0	.9 ± 0	.9 ± 0

[1] The test diets used yolk powders prepared from eggs from laying hens fed diets containing 10% full-fat flax (Hn-3), 12% sunflower seed (Hn-6), or soybean meal plus animal tallow (CON). Female Sprague-Dawley rats were fed for a period of 28 days (Mean ± SEM, n=7).

Table 6.5 Rat performance and the size of liver and heart tissues of rats in Experiment 2

	Diet[1]		
	Hn-3	Hn-6	Hn-9
Initial body wt (g)	127.0 ± 3.0	130.0 ± 2.5	124.7 ± 2.1
Final body wt (g)	237.4 ± 3.7	229.9 ± 6.6	230.9 ± 9.8
Feed consumption (g/rat/day)	17.0 ± .5	16.7 ± .5	16.6 ± .9
Liver tissue (g)	8.3 ± .3	8.2 ± .4	8.5 ± .7
Heart tissue (g)	.9 ± 0	.8 ± .1	.9 ± .1

[1] Te test diets contained yolk powders prepared from eggs laid by laying hens fed diets containing 15% full-fat flax seed (Hn-3), 21% regular high linoleic acid sunflower seed (Hn-6), or 18% high oleic acid sunflower seed (Hn-9). Female Sprague-Dawley rats were fed for a period of 31 days (Mean ± SEM, n = 10).

Table 6.6. The HMG CoA reductase (HMGR) activity of liver tissue and the production of prostaglandin E_2 (PG-E_2) by rat epitrochlearis muscle in Experiment 2

	Test diet[1]		
	Hn-3	Hn-6	Hn-9
HMGR activity[2] (pmol/mg/min)	15.2 ± 2.3^a	12.0 ± 2.2^a	17.8 ± 3.4^a
PG-E_2 synthesis[2] (pg/mg/hr)	$6.2 \pm .7^b$	13.3 ± 2.7^a	$8.3 \pm .7^{ab}$

[a,b] Means in a row without common letters differ significantly ($P<.05$).

[1] The rat test diets contained yolk powders prepared from eggs laid by laying hens fed diets containing full-fat flax seed (Hn-3), regular high linoleic acid sunflower seed (Hn-6), or high oleic acid sunflower seed (Hn-9). Data were presented as Mean ± SEM, n = 10.

[2] The activity of HMGR was presented as pmol of products formed per mg of hepatic microsome proteins per min. The rate of PG E_2 synthesized by rat epitrochlearis muscle was expressed as pg of PG E_2 released into incubate medium per mg of muscle per hr.

Table 6.7. Major n-3 and n-6 fatty acids of rat liver total lipids at the end of the feeding trial of Experiment 1

Fatty acid	Dietary treatment[1]		
	Hn-3	Hn-6	CON
	% of total fatty acids		
18:2(n-6)	9.5 ± 0.5^b	13.8 ± 0.9^a	8.0 ± 0.3^c
20:4(n-6)	8.8 ± 1.1^a	9.6 ± 1.4^a	9.7 ± 0.2^a
18:3(n-3)	1.8 ± 0.3^a	0.3 ± 0^b	0.3 ± 0^b
20:5(n-3)	1.1 ± 0.3^a	0.1 ± 0^b	0.2 ± 0^b
22:5(n-3)	0.5 ± 0.1^a	0.1 ± 0^b	0.1 ± 0^b
22:6(n-3)	4.8 ± 0.8^a	2.0 ± 0.3^b	2.4 ± 0.4^b

[a-c]: Means in a row without common superscripts differ significantly ($P<0.05$).

[1] Data were presented as Mean ± SEM, n=7. The test diets used yolk powders prepared from eggs of laying hens fed diets containing 10% full-fat flax (Hn-3), 12% sunflower seed (Hn-6), or soybean meal plus animal tallow (CON).

Table 6.8. Major fatty acids of liver phospholipids of rats in Experiment 1

Fatty acid	Dietary Treatments[1]		
	Hn-3	Hn-6	CON
	% of total fatty acids		
16:0	16.4 ± 1.4^{ab}	17.1 ± 0.7^{a}	15.7 ± 0.6^{b}
18:0	27.5 ± 1.9^{a}	26.6 ± 1.7^{a}	27.5 ± 1.6^{a}
18:1	15.0 ± 3.0^{a}	16.7 ± 2.5^{a}	15.9 ± 2.3^{a}
18:2(n-6)	7.5 ± 0.9^{a}	7.8 ± 0.6^{a}	6.2 ± 1.0^{b}
18:3(n-3)	0.4 ± 0.3^{a}	0.5 ± 0.3^{a}	0.3 ± 0.3^{a}
20:4(n-6)	19.8 ± 2.9^{b}	21.2 ± 2.1^{ab}	24.1 ± 2.3^{a}
20:5(n-3)	0.9 ± 0.3	0	0
22:6(n-3)	7.9 ± 1.2^{a}	5.0 ± 1.1^{b}	5.6 ± 0.8^{b}
20:4(n-6)/ (20:5+22:6)(n-3)	2.25	4.24	4.30

[a-c]: Means in a row without common superscripts differ significantly (P<.05).

[1] Data were presented as Mean ± SEM, n=7. The test diets used yolk powders prepared from eggs of laying hens fed diets containing 10% full-fat flax (Hn-3), 12% sunflower seed (Hn-6), or soybean meal plus animal tallow (CON).

Table 6.9. Major fatty acids of plasma total lipids of rats in Experiment 2

	Test diet[1]		
	Hn-3	Hn-6	Hn-9
	% of total fatty acids		
C16:0	$17.2 \pm .1^{a}$	$17.7 \pm .2^{a}$	$17.6 \pm .4^{a}$
C18:0	$13.1 \pm .1^{a}$	$15.0 \pm .4^{a}$	$13.5 \pm .5^{a}$
C16:1	$3.7 \pm .2^{a}$	$3.0 \pm .1^{a}$	$3.2 \pm .1^{a}$
C18:1	$31.3 \pm .5^{a}$	$26.0 \pm .7^{b}$	32.1 ± 1.0^{a}
C18:2 n-6	$13.9 \pm .5^{ab}$	$14.9 \pm .2^{a}$	$11.8 \pm .3^{b}$
C18:3 n-3	$6.2 \pm .1^{a}$	$4.3 \pm .1^{b}$	$4.7 \pm .2^{b}$
C20:4 n-6	$11.8 \pm .3^{b}$	$18.4 \pm .8^{a}$	16.1 ± 1.0^{a}
C20:5 n-3	1.0^{a}	0^{b}	0^{b}
C22:6 n-3	1.9 ± 0^{a}	$.8 \pm .2^{b}$	$1.0 \pm .2^{b}$
Σ MUFA	35.0	29.0	35.3
Σ n-6 PUFA	25.7	33.3	27.9
Σ n-3 PUFA	9.1	5.1	5.7
Σ(n-6)/Σ(n-3) ratio	2.8	6.5	5.5

[a,b] Means in a row without common letters differ significantly ($P<.05$).

[1] The rat test diets contained yolk powders prepared from eggs laid by laying hens fed diets containing full-fat flax seed (Hn-3), regular high linoleic acid sunflower seed (Hn-6), or high oleic acid sunflower seed (Hn-9). Data were presented as Mean ± SEM, n = 5 pooled plasma samples of two rats each. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 6.10. Major fatty acids of liver phosphatidylcholine fraction of rats in Experiment 2

	Test diet[1]		
	Hn-3	Hn-6	Hn-9
	% of total fatty acids		
C16:0	16.5 ± 1.2[a]	15.3 ± .5[a]	16.1 ± .9[a]
C18:0	22.2 ± .8[b]	26.4 ± .5[b]	33.9 ± 2.2[a]
C16:1	1.3 ± .1[a]	1.4 ± .1[a]	.8 ± .1[a]
C18:1	14.8 ± 1.2[a]	14.5 ± .5[a]	9.2 ± .5[b]
C18:2 n-6	7.2 ± .4[a]	5.8 ± .0[b]	4.3 ± .4[b]
C18:3 n-3	.9 ± .2[a]	.9 ± .1[a]	.4 ± .1[a]
C20:4 n-6	19.0 ± 1.0[b]	23.8 ± 1.0[a]	29.2 ± 2.3[a]
C20:5 n-3	1.3 ± .1[a]	.1[b]	0[b]
C22:6 n-3	6.3 ± .8[a]	4.9 ± .3[a]	4.8 ± .3[a]
Σ MUFA	16.1	15.9	10.0
Σ n-6 PUFA	26.2	29.6	33.5
Σ n-3 PUFA	8.6	5.9	5.3
Σ(n-6)/Σ(n-3) ratio	2.8	5.0	6.3

[a,b] Means in a row without common letters differ significantly (P<.05).

[1] The rat test diets contained yolk powders prepared from eggs laid by laying hens fed diets containing full-fat flax seed (Hn-3), regular high linoleic acid sunflower seed (Hn-6), or high oleic acid sunflower seed (Hn-9). Data were presented as Mean ± SEM, n = 5 pooled samples of two each. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 6.11. Major fatty acids of liver phosphatidylethanolamine fraction of rats in Experiment 2

	Test diet[1]		
	Hn-3	Hn-6	Hn-9
	% of total fatty acids		
C16:0	15.3 ± .8^{a}	15.3 ± .4^{a}	15.4 ± .6^{a}
C18:0	27.8 ± 1.1^{a}	28.4 ± 1.1^{a}	29.0 ± .7^{a}
C16:1	1.3 ± .1^{a}	1.0 ± .1^{a}	1.0 ± .1^{a}
C18:1	12.7 ± 1.2^{a}	11.5 ± 1.0^{a}	14.2 ± 1.6^{b}
C18:2 n-6	5.9 ± .2^{a}	4.7 ± .0^{a}	5.0 ± .4^{a}
C18:3 n-3	1.2 ± .2^{a}	.9 ± .1^{a}	1.1 ± .2^{a}
C20:4 n-6	19.4 ± .8^{b}	24.6 ± 1.1^{a}	24.3 ± 1.8^{a}
C20:5 n-3	2.4 ± .1^{a}	.1^{b}	0^{b}
C22:6 n-3	10.4 ± .6^{a}	3.7 ± .6^{b}	6.4 ± 1.0^{b}
Σ MUFA	14.0	12.5	15.3
Σ n-6 PUFA	25.3	29.3	29.3
Σ n-3 PUFA	14.7	4.7	7.5
Σ(n-6)/Σ(n-3) ratio	1.7	6.2	3.9

[a,b] Means in a row without common letters differ significantly (P<.05).

[1] The rat test diets contained yolk powders prepared from eggs laid by laying hens fed diets containing full-fat flax seed (Hn-3), regular high linoleic acid sunflower seed (Hn-6), or high oleic acid sunflower seed (Hn-9). Data were presented as Mean ± SEM, n = 5 pooled samples of two each. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

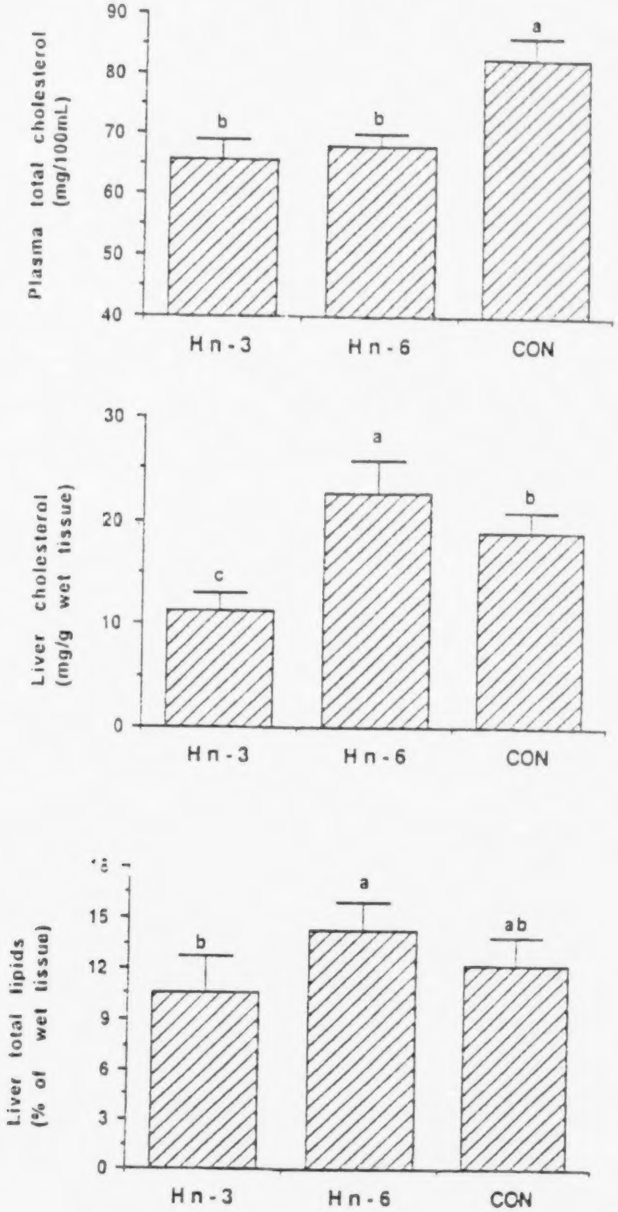

Figure 6.1. Plasma and liver cholesterol levels and liver total lipid content of rats at the end of 28-day feeding trial Experiment 1 (Mean ± SEM, n=7). The test diets used yolk powders prepared from eggs laid by laying hens fed diets containing 10% flax seed (Hn-3), 12% sunflower seed (Hn-6), or animal tallow control (CON). Bars without a common letter differ significantly (P<.05).

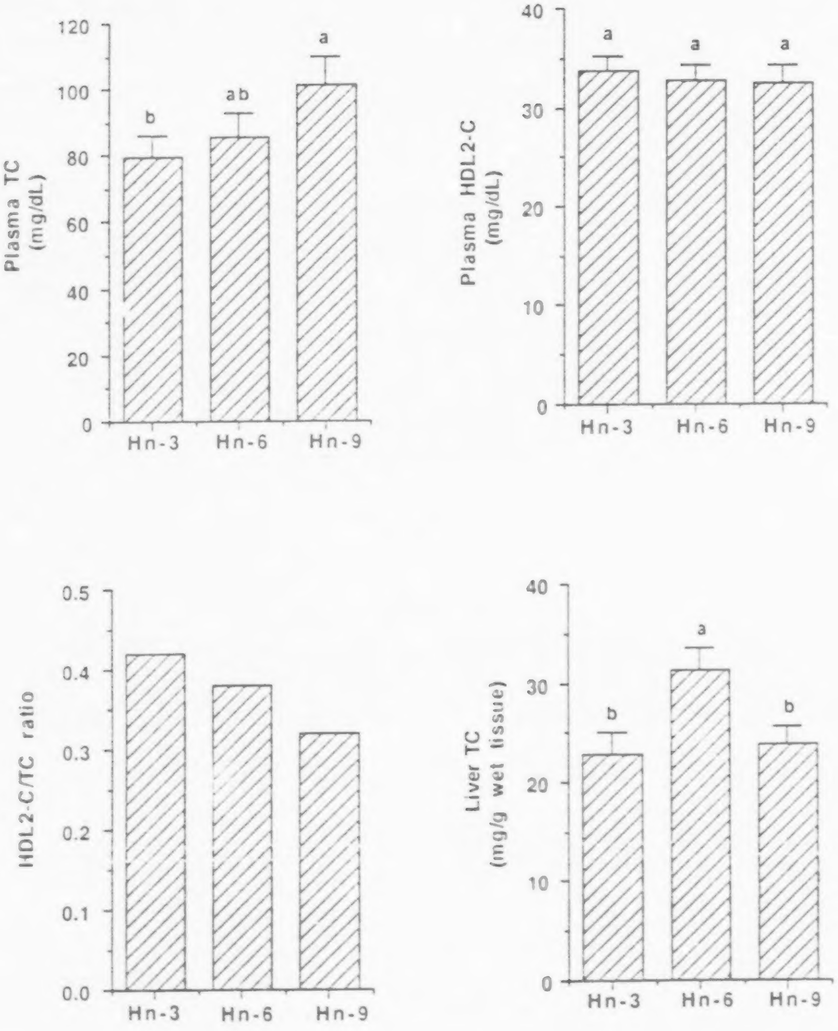

Figure 6.2. Plasma total (TC), high-density lipoprotein (HDL_2-C) cholesterol, and the ratio of HDL_2-C/TC, and liver cholesterol levels of rats at the end of 31-day feeding trial Experiment 2 (Mean ± SEM, n=10). The test diets used yolk powders prepared from eggs laid by laying hens fed diets containing 15% flax seed (Hn-3), 18% regular high linoleic acid sunflower seed (Hn-6), or 21% high oleic acid sunflower seed (Hn-9). Bars without a common letter differ significantly (P<.05).

6.5. References

Al-Shurbaji, A., C. Larsson-Backstrom, L. Berglund, G. Eggertsen, and I. Bjorkhem, 1991. Effect of n-3 fatty acids on the key enzymes involved in cholesterol and triglyceride turnover in rat liver. Lipids 26:385-389.

Aukeman, H. M., and B. J. Holub, 1989. Effect of dietary supplementation with a fish oil concentrate on the alkenylacyl class of ethanolamine phospholipid in human platelets. J. Lipid Res. 30:59-64.

Bang, H. O., J. Dyerberg, and H. M. Sinclair, 1980. The composition of the eskimo food in Greenland. Am. J. Clin. Nutr. 33:2657-2661.

Bartov, I., R. Reiser, and G. R. Henderson, 1973. Hypercholesterolemic effect in the female rat of egg yolk versus crystalline cholesterol dissolved in lard. J. Nutr. 103:1400-1405.

Bloomfield, D. K., 1964. Cholesterol metabolism III. Enhancement of cholesterol absorption and accumulation in safflower oil-fed rats. J. Lab. Clin. Med. 64:613-623.

Brenner, R. R., 1981. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog. Lipid Res. 20:41-50.

Brown, M. S., J. L. Goldstein, and J. M. Dietschy, 1979. Active and inactive forms of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the liver of the rat. J. Biol. Chem. 254:5144-5149.

Budowski, P., and M. A. Crawford, 1986. Effect of dietary linoleic and alpha-linolenic acids on the fatty acid composition of brain lipids in the young chick. Prog. Lipid Res. 25:615-618.

Clifford, A. J., L. M. Smith, R. K. Creveling, C. L. Hamblin, and C. K. Clifford, 1986. Effects of dietary triglycerides on serum and liver lipids and sterol excretion of rats. J. Nutr. 116:944-956.

Fenton, M., and J. S. Sim, 1991. Simple and accurate determination of egg yolk cholesterol content by on-column capillary gas chromatography. J. Chromatography 540:323-329.

Folch, J., M. Lees, G. H. Sloan-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Garg, M. L., A. A. Wierzbicki, A. B. R. Thomson, and M. T. Clandinin, 1989. Dietary saturated fat level alters the competition between α-linolenic and linoleic acid. Lipids 24:334-339.

Garg, M. L., and J. R. Sabine, 1988. Homeostatic control of membrane cholesterol and fatty acid metabolism in the rat liver. Biochem. J. 251:11-16.

Garg, M. L., E. Sebokova, A. Wierzbicki, A. B. R. Thomson, M. T. Clandinin, 1988. Differential effects of dietary linoleic and α-linolenic acid on lipid metabolism in rat tissues. Lipids 23:847-852.

Grundy, S. M., 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N. Engl. J. Med. 314:745-748.

Hartree, E. F., 1972. Determination of protein: a modification of the Lowery method that gives a linear photometric response. Anal. Biochem. 48:422-327.

Health and Welfare Canada, 1990. Nutrition Recommendations. Canadian Government Publishing Centre, Ottawa, Canada.

Jimenez, M. A., M. L. Scarino, F. Vignolini, and E. Menghert, 1990. Evidence that polyunsaturated lecithin induces a reduction in plasma cholesterol level and favorable changes in lipoprotein composition in hypercholesterolemic rats. J. Nutr. 120:659-667.

Kellogg, T. F., 1974. Steroid balance and tissue cholesterol accumulation in germfree and conventional rats fed diets containing saturated an polyunsaturated fats. J. Lipid Res. 15:574-579.

Kinsella, J. E., B. Lokesh, and R. A. Stone, 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52:1-28.

Kris-Etherton, P. M., C. Y. Ho, and M. A. Fosmire, 1984. The effect of dietary fat saturation on plasma and hepatic lipoproteins in the rat. J. Nutr. 114:1675-1682.

Mahfouz, M. M., T. L. Smith, and F. A. Kummarow, 1984. Effects of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat liver microsomes. Lipids 19:214-222.

Metcalfe, L. D., A. A. Schmitz, and J. B. Pelka, 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem. 33:363-364.

Nestel, P. J., 1986. Fish oil attenuates the cholesterol induced rise in lipoprotein cholesterol. Am. J. Clin. Nutr. 43:752-757.

O'Brien, B. C., and S. M. Corrigan, 1988. Influence of dietary soybean and egg lecithins on lipid responses in cholesterol-fed guinea-pigs. Lipids 23:647-650.

Olomu, J. M., and V. E. Baracos, 1991. Prostaglandin synthesis and fatty acid composition of phospholipids and triglycerides in skeletal muscle of chicks fed combinations of flaxseed oil and animal tallow.

Lipids 26:743-749.

SAS Institute, 1985. SAS^R/STAT Guide for Personal Computers, 6th ed. SAS Institute, Inc., Cary, NC.

Sim, J. S., S. O. Chung, P. A. Borgersen, Z. Jiang, and W. P. Chorney, 1991a. Cholesterol and fatty acid compositions of commercially produced Alberta eggs (A provincial-wide survey study). Pages 20-21 in: 70th Feeders' Day Report, University of Alberta.

Sim, J. S., E. Nwokolo, Z. and Jiang, 1991b. Modulation of plasma and tissue cholesterol and fatty acid composition by feeding flax and canola seeds and oils to rats. Can. J. Animal Sci. (in press).

Simopoulos, A. P., 1989. Summary of the NATO advanced research workshop on dietary omega-3 and omega-6 fatty acids: biological effects and nutritional essentiality. J. Nutr. 119, 521-528.

Simopoulos, A.P., 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54:438-463.

Singer, P., I. Berger, M. Wirth, W. Godicke, W. Jaeger, and S. Voight, 1986. Slow desaturation and elongation of linoleic and linolenic acid as a rationale of eicosapentaenoic acid-rich diet to lower blood pressure and serum lipids in norma, hypertensive and hyperlipidemic subjects. Prostagl. Leuko. Med. 24:173-193.

Sjoblom, L., and A. Eklund, 1989. Determination of HDL_2 cholesterol by precipitation with dextran sulfate and magnesium chloride: establishing optimal conditions for rat plasma. Lipids 24:532-534.

Sugano, M., M. Watanabe, M. Kohno, Y. J. Cho, and T. Ide, 1983. Effects of dietary trans-fat on billiary and fecal steroid excretion and serum lipoproteins in rats. Lipids 18:375-381.

Terpstra, A. H. M., G. van Tintelen, and C. E. West, 1982. The effect of semipurified diets containing different proportions of either casein or soybean protein on the concentration of cholesterol in whole serum, serum lipoproteins and liver in male and female rats. Atherosclerosis 42:85-95.

Chapter 7. Fatty Acid Modified Eggs as Human Food:

Cholesterolemic Effects and Overall Quality

7.1. Consumption of n-3 PUFA Enriched Eggs and Changes in Blood Lipids in Healthy Volunteers[1]

7.1.1. Introduction

Several lines of information suggest that humans evolved on a diet that was low in fat and saturated fatty acids, and a ratio of n-6 to n-3 polyunsaturated fatty acids (PUFA) about 1:1 (Eaton and Konner, 1985; Leaf and Weber, 1987; Simopoulos, 1990). Modern agribusiness and food industry were blamed for bringing about abrupt changes in the quantity and quality of dietary fat. As a result, today's diet is high in total fat and saturated fat with a ratio of n-6 to n-3 PUFA of 10:1 to 25:1 (Simopoulos, 1991). Many of the food animal products, such as pork, beef, fish, and eggs contain less n-3 PUFA than those from the wild animals (Simopoulos, 1991). According to the diet-heart hypothesis, the amount and the type of dietary lipids play important roles in influencing the plasma and lipoprotein lipid levels, which, in turn, were associated with the risk of coronary heart disease (CHD) (US Dep't Health & Human Services, 1988). It is generally accepted that replacing dietary saturated fat with polyunsaturated fat will reduce the atherogenic plasma total (TC) and low-density lipoprotein cholesterol (LDL-C) levels (US Dep't Health & Human Services, 1988). The significance of n-3 polyunsaturated fatty acids (n-3 PUFA) in the amelioration and prevention of atherosclerosis and other diseases has been appreciated only in the past decade (Dyerberg et al., 1978; Dyerberg and Bang, 1979). The beneficial effects of dietary n-3 PUFA include, among others, the reduction of plasma triglyceride (TG) concentration (Connor, 1986; Harris, 1989), blood pressure (Knapp, 1989), platelet aggregation (Von Shacky et al., 1985), and tumor growth (Karmali et al., 1984). Because of the competition of n-6 and n-3 PUFA for the enzymes involved in further metabolism, in eicosanoid production, and in incorporation into tissue lipids, the ratio of dietary n-6 to n-3 PUFA has been suggested to be more important than the absolute amount of fat and the ratio should be reduced (Neuringer and Connor, 1986; Health and Welfare Canada, 1990). It should be up to today's food producers to play an active role in balancing the fatty acid composition of their products. Indeed, efforts have been made during the past several years to modify the fatty acid composition of the food animal products (National Research Council (US), 1988).

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Chicken eggs, due to their relatively high cholesterol content, have been singled out by the diet-heart advocators to avoid (Connor and Connor, 1983) even though the egg contains the best and least-expensive high quality protein and a balanced distribution of minerals and vitamins (Shrimpton, 1987). Various attempts to reduce egg cholesterol content through genetic selection and diet and drug treatments were not successful (Naber, 1983). However, it is known that the fatty acid composition of the yolk can be easily modified through alteration of the dietary fat of laying hens (Cruickshank, 1934). The contents of n-3 PUFA, such as α-linolenic (LNA), docosapentaenoic (DPA), and docosahexaenoic acids (DHA) of the yolk were significantly increased by feeding laying hens diets containing canola or flax seeds or oils (Sim et al., 1988; Caston and Leeson, 1990), or fish oil (Yu and Sim, 1987; Hargis et al., 1991). The deposition of LNA was found to be exclusively in yolk triglycerides, while the longer chain EPA and DHA were preferentially incorporated in yolk phospholipids, particularly in the phosphatidylethanolamin (Jiang et al., 1991). Feeding the n-3 PUFA-enriched eggs to rats significantly reduced both plasma and liver cholesterol contents when compared to the regular egg feeding (Jiang and Sim, 1992). The synthesis of prostaglandin E_2 by the epitrochlearis muscle was also inhibited by feeding rats the n-3 PUFA eggs (previous chapter). More recently, Oh et al. (1991) reported that in a cross-over study, consumption of four DHA-enriched eggs per day for 4 wk prevented plasma TC from rising. In the present study, we examined the effect of consumption of eggs that were enriched predominately with LNA on the plasma lipids in healthy volunteers.

7.1.2. Subjects and methods

Twenty-four healthy male students aged 18-32 were recruited from the University. The selected subjects were not taking medications known to affect lipid metabolism. Each subject was requested to sign a consent form. The consent form and procedures followed were approved by the Ethics Committee on Human Subjects for Research at the University of Alberta. The personal data of all subjects are presented in Table 7.1.

The subjects were randomly divided into two groups with twelve each.

Breakfast, consisting of two fried eggs (n-3 PUFA for Group A and regular for Group B), two pieces of 60% whole wheat bread, jams and apple or orange juice, were served between 0730 to 0900 each weekday morning in the Food Science Department at the University of Alberta. The subjects took home the eggs for the weekend and holidays and were requested to cook the egg without using cooking oil or butter. During the study period, subjects were encouraged to maintain their body weight and habitual diet, but avoid excessive consumption of organ meat, fish, and alcohol. One subject from Group A dropped out of the study due to personal reasons. The major fatty acid composition of n-3 PUFA enriched and regular eggs was determined by the gas chromatographic technique (Jiang et al., 1991) and is presented in Table 7.2.

Before and at the end of the 18 day study, subjects were fasted for more than 12 h, and blood samples were drawn from an antecubital vein into evacuated tubes containing 0.1% EDTA. Plasma was separated by centrifugation. The high-density lipoprotein fraction was separated by precipitation and removal of low-density and very-low-density lipoproteins (LDL and VLDL) by phosphotungstic acid/magnesium chloride (HDL Reagent, Hoffmann-La Roche Limited, Etobiocoke, Ontario, Canada). Plasma total and HDL cholesterol (TC and HDL-C) were determined enzymatically using a cholesterol assay kit (Diagnostic Chemicals Limited, Charlottetown, PEI, Canada). Plasma triglyceride (TG) was assayed using a diagnostic kit (Diagnostic Chemicals Limited, Charlottetown, PEI, Canada). Plasma LDL cholesterol was calculated from plasma TC, HDL-C, and TG levels according to the Friedewald equation (Masse, 1991). These lipid analyses were carried out by an independent commercial clinical laboratory (Dr. T A

Kasper's Laboratory, Edmonton, Alberta, Canada).

Plasma total lipids were extracted according to Folch et al. (Folch et al., 1957). The chloroform extracts were dried under nitrogen, and methylated according to Metcalfe et al. (1961) using Boron-trifluoride. The fatty acid methyl esters were separated and quantified on a Varian 3700 gas chromatograph as described previously (Jiang et al., 1991).

Paired t tests were used to compare the changes of examined parameters before and at the end of the experiment (Steel and Torrie, 1980).

7.1.3. Results

The effects of consuming two n-3 PUFA-enriched (Group A) or regular eggs (Group B) per day on plasma and lipoprotein lipids of individual subjects were shown in Fig. 7.1. The mean changes of each group in plasma and lipoprotein lipids brought about by egg consumption were also depicted in Fig. 7.2 for direct comparison. Consuming two regular eggs a day for a period of 18 days tended to increase plasma TC level, elevated LDL-C significantly (P<.05), but did not change plasma HDL-C nor plasma TG levels. On the other hand, those who consumed the n-3 PUFA-enriched eggs maintained their initial plasma TC and LDL-C levels, elevated HDL-C (P<.05), and reduced plasma TG by more than 40% (P<.01).

Fig. 7.1 also showed the changes in plasma TC and HDL-C of individual subjects who, according to the Canadian Consensus Conference on Cholesterol (1988), had undesirably high TC (>4.60 mmol/L) or low HDL-C (<.89 mmol/L) before the experiment. Plasma TC levels of all three subjects from Group A were reduced to less than 4.60 mmol/L while that of one subject from Group B was not changed. One subject from each group had initial HDL-C lower than .89 mmol/L. At the end of the experiment, however, HDL-C of Group A subject was increased by more than 30% (from .74 to .97 mmol/L), while the Group B subject still had HDL-C lower than 0.89 mmol/L (from .83 to .86 mmol/L).

Changes in HDL-C/TC and HDL-C/LDL-C ratios in individual subjects before and after egg consumption were presented in Fig. 7.3. Both ratios were increased in subjects who consumed n-3 PUFA enriched eggs, and the increase of HDL-C/TC was statistically significant ($P<.01$). On the other hand, both ratios were decreased in subjects who consumed regular eggs. The reduction of HDL-C/LDL-C was significant ($P<.05$).

The composition of plasma major fatty acids is shown in Table 7.3. Overall, the percentages of plasma n-3 PUFA were increased in subjects consuming n-3 PUFA eggs (Group A). Alpha-linolenic acid increased most, followed by DHA and EPA. Consequently, the ratio of n-6 to n-3 PUFA decreased in subjects of Group A. No such changes were found in those who consumed regular eggs (Group B).

7.1.4. Discussion

The present study clearly demonstrated that the influence of egg consumption on plasma and lipoprotein lipids in human subjects can be altered by modifying the fatty acid composition of yolk lipids. The eggs used in this study were enriched mainly with alpha-linolenic acid, but considerable amounts of longer chain n-3 PUFA, such as DHA, DPA, and EPA, were also incorporated into yolk phospholipids (Jiang et al., 1991). One large n-3 PUFA egg would supply about 500 mg of LNA, and 100 mg of DHA, DPA, and EPA. This modification of yolk fatty acid composition led to a significant reduction in the n-6/n-3 PUFA ratio in n-3 enriched eggs, particularly when compared to supermarket eggs. The n-6/n-3 ratio of commercially produced eggs is in the range of 15 to 20 (Sim et al., 1991; Simopoulos, 1991).

A questionire indicated that subjects participated in this study consumed between 0 to 2 eggs per week before the experiment. Consuming two eggs a day would add another 440 mg of cholesterol to their habitual daily intake of 440 mg (Health and Welfare Canada, 1990). This would elevate the plasma TC by .19 mmol/L according to Key's equation (Keys, 1984), assuming that the daily energy and initial cholesterol intakes were 3000 kcal and 440 mg per person per day, respectively, and there were no changes in dietary fat. The plasma TC levels of subjects who consumed regular eggs (Group B) were indeed increased by .21 mmol/L by the end of the experiment (Fig. 7.1). The elevation of cholesterol was almost exclusively in the LDL fraction, resulting in statistical significant increase of LDL-C ($P < .05$). These results confirmed previous reports that consuming two or more eggs a day in addition to the habitual diet significantly increased plasma LDL-C, while the increase of plasma TC might be insignificant (Applebaum-Bowden et al., 1984).

Plasma TC and LDL-C of those who consumed n-3 PUFA eggs, however, were virtually unaffected. Thus consumption of the n-3 PUFA eggs were able to attenuate the effects of increased cholesterol intake on plasma TC and LDL-C. These results are consistent with those in animal experiments which demonstrated that feeding n-3 PUFA enriched eggs to rats significantly reduced plasma TC when compared to regular eggs (Jiang and Sim, 1992) and those of Oh et al. (1991). The observed hypocholesterolemic effects could be attributed to a higher n-3 PUFA content in these eggs. Dietary LNA was reported to be more potent than linoleic acid in lowering plasma cholesterol level in rats (Garg et al., 1988). The effects of dietary cholesterol on plasma lipids were also reported to be attenuated by dietary LNA in rats (Kritchevsky et al., 1991) and by longer chain n-3 PUFA in human subjects (Nestel, 1986). The considerable amount of longer chain n-3 PUFA accumulated in the yolk, such as DHA and DPA, might also attribute to the cholesterol lowering effects of the n-3 PUFA egg, since the longer chain n-3 PUFA were reported to be more potent than LNA in reducing plasma lipids (Nestel, 1986; Singer et al., 1986).

The significant elevation of HDL-C in subjects who consumed n-3 PUFA eggs, but not in those who had regular eggs, was consistent with the findings of many clinical trials demonstrating that dietary n-3 PUFA increased HDL-C (von Losonczy et al., 1978; Sanders and Roshannai, 1983; Saynor et al., 1984). However, the dietary effects of n-3 PUFA on HDL-C may also depend upon other dietary lipids, including cholesterol. Several trials have consistently demonstrated that dietary cholesterol from egg consumption increased both total HDL and HDL_2 cholesterol (see reviews by Pyorala, 1987; Grundy et al., 1988), leading to a paradox that a high intake of cholesterol would seemingly protect against atherosclerosis. There might exist an interaction between dietary n-3 PUFA and cholesterol which could influence HDL metabolism in a distinctive way.

As the role of HDL particles in protection against atherosclerosis gains more support by recent studies, it has been suggested that the ratio of HDL-C to TC or HDL-C to LDL-C could be a more powerful indicator for predicting CHD (Gordon et al., 1977; Goldbourt et al., 1985). The elevation of both HDL-C/TC and HDL-C/LDL-C ratios by n-3 PUFA egg consumption might thus be of particular importance for the consumer. On the other hand, the reduction of both HDL-C/TC and HDL-C/LDL-C ratios in those who consumed regular eggs might be undesirable in terms of CHD risk.

The most drastic changes in plasma and lipoprotein lipids in the present study was the reduction of plasma TG in those who consumed n-3 PUFA eggs. Dietary n-3 PUFA have been reported to preferentially depress plasma TG (Connor, 1986). The maximal TG lowering in normal human subjects was reported to be at a dose of 4.5 g/day n-3 PUFA (Harris et al., 1988; Harris, 1989). The present study demonstrated that even at a dose as low as 1.2 g/d, dietary n-3 PUFA was associated with a profound depression of plasma TG levels in normal subjects.

The plasma fatty acid composition of those who consumed n-3 PUFA eggs was also moderately enriched with n-3 PUFA, particularly with LNA and DHA, and the ratio of n-6/n-3 reduced. It has been reported that the incorporation of n-3 PUFA into plasma lipids occurs rapidly in a dose-dependent manner with dietary n-3 PUFA (Von Shacky et al., 1985). The incorporation of n-3 PUFA into tissue lipids and the reduction of n-6/n-3 ratio might affect the quantity and profiles of eicosanoid production (Kinsella et al., 1990) in such a manner as to be ameliorative against atherosclerosis.

In the Western World, animal products contribute more than 60% of total lipids, and 70% of saturated fats (National Research Council, 1988) of the diet. Consumers' preference for the animal products will likely continue. Thus, it would be of national strategic importance in the fight against CHD to design and modify animal products in such a way that the dietary risks of CHD would be minimized. Both the epidemiological (Kromhout et al., 1985) and clinical intervention (Burr et al., 1989) studies have demonstrated a decrease of CHD mortality in people consuming relatively small amounts of n-3 PUFA (.5 g/d) over a long period of time. Although one large n-3 PUFA enriched egg used in the present study will provide only .6 g n-3 PUFA, it would still have beneficial effects for egg consumers. Similar efforts have also been made to modify meat products (Ajuyah et al., 1991) to achieve the ultimate goal of minimizing the dietary risk of CHD.

7.2. Effects of Yolk Fatty Acid Modification on the Internal and Sensory Qualities of the Egg[1]

7.2.1. Introduction

Attempts to reduce cholesterol content of eggs by dietary manipulations were not successful (Naber, 1983). It is known, however, that the fatty acid composition of yolk lipids can be readily altered by modifying the quantity and type of fat in laying hen diets (Cruickshank, 1934; Noble, 1987). There is a growing consensus among human nutritionists that dietary n-3 polyunsaturated fatty acids (n-3 PUFA), such as α-linolenic acid (LNA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), play important roles in human health (Kinsella et al., 1990).

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[1] A version of this section has been published. Jiang, Z., D. U. Ahn, L. Ladner, and J. S. Sim, 1992. Poultry Science 71:378-382.

The cholesterol-lowering and ameliorative effects against atherosclerosis of dietary n-3 PUFA are of particular interest to egg producers, consumers, and researchers. These much needed n-3 PUFA could be readily incorporated into yolk lipids by feeding laying hen diets containing full-fat flax or canola seed (Nwokolo et al., 1988; Sim, 1990; Caston and Leeson, 1990) or fish oils (Yu and Sim, 1987; Hargis et al., 1991). Recent studies from our laboratory demonstrated that longer chain n-3 PUFA (EPA, DHA, and docosapentaenoic acid) were preferentially deposited into yolk phosphatidylethanolamine fraction after feeding flax seeds (Jiang et al., 1991). Feeding n-3 PUFA-enriched eggs to rats reduced plasma and liver cholesterol contents, indicating that the cholesterolemic properties of chicken eggs were markedly modified by incorporating n-3 PUFA into yolk lipids (Jiang and Sim, 1992). The n-3 PUFA-enriched eggs might thus be more appealing to health conscious consumers.

Consumer acceptance of eggs, however, also depends on egg quality parameters such as Haugh units, yolk color, storage stability, and in particular the sensory traits. There is little information on the effects of dietary full fat oil seeds or fish oils on these quality parameters. The objective of the current study was to examine the dietary effects of n-3 or n-6 PUFA rich oil seeds on the sensory quality of hard-cooked eggs and the internal quality of eggs during storage.

7.2.2. **Materials and Methods**

A total of 528 Single Comb White Leghorn laying hens, 16 mo of age, were housed in double deck cage batteries with two birds in each cage (.31

x .36 m). The birds were allotted to one of the four dietary treatments with each treatment replicated three times randomly among the three batteries. Four isonitrogeneous, isocaloric laying hen diets were prepared to contain either 15% full fat flax (Hn-3), 18% high oleic acid sunflower seed (Hn-9), 21% regular high linoleic acid sunflower seed (Hn-6), or 3% animal tallow (Control) (Table 5.1, chapter 5 of this thesis). These diets provided 15.0% CP, 3.6% calcium, and 2,728 kcal ME/kg. Eggs were collected during the 4th wk of the feeding trial and stored at 4 C. On Day 0, 14, 28, and 42 of storage, eight eggs were selected randomly from each replicate making a total of 24 eggs per treatment. Haugh units were determined according to Haugh (1937) and the yolk color index was obtained by comparison to the Roche Yolk Color Fan (Vuilleumier, 1969). Two yolk samples from each replicate were subjected to fatty acid analyses by gas chromatography as described previously (Jiang et al., 1991).

On Day 7 of storage, 2 doz eggs from each treatment were sampled for sensory evaluation. Two separate studies were conducted, one at the University and the other at the Food Processing Development Centre of Alberta Agriculture, Leduc, Alberta, Canada. In the University study, 28 untrained students and staff members from the Departments of Food Science and Animal Science participated. The study by the Food Centre was conducted using 12 trained panellists (five males and seven females, age 31 to 45) with three repetitions for a total of 36 observations. In both studies, eggs were cooked by bringing tap water to a boil and then kept simmering for 20 min. After being cooled in tap water for 15 min, eggs were shelled and cut into four pieces along the major axis. Four samples were coded using three-digit random numbers and presented randomly to panellists. Cold tap water was provided for panellists to rinse their mouths between samples. The evaluations were carried out in sensory evaluation rooms with a dim red light to eliminate any possible effects of sample color on the preference test. Nine-cm hedonic lines were used for panellists to quantitatively record their preference scores. Each line was labelled with descriptors "Dislike extremely", "Neither like nor dislike", and "Like extremely" at the left end, middle, and the right end of the line, respectively. The preference score was measured by the distance from the left end to where it was checked. Thus a score of 0 represented "dislike extremely", and a 9 "like extremely". The panellists in the University study were asked to describe any off-flavors of the egg samples. Characteristic descriptors for fresh egg flavors such as "sweet", "acid", "sulfury", "salty", "earthy", "bitter", "fatty", "hydrolyzed protein" (Koehler and Jacobson, 1966), and "fishy" were provided for the panellists in the Food Centre study.

Two-way ANOVA was used to analyze the effects of diet and storage on the Haugh units and the yolk color index using eggs within diet by storage as the error term. Means of main effects (diet and storage) were separated by the Student-Newman-Keuls (SNK) test. Means of interaction (diet by storage) were compared using the PDIFF function of the SAS program (SAS Institute, 1985). Sensory evaluations were analyzed by one-way ANOVA with dietary treatments as the main effects and individual scores within treatment used as the error term. Treatment means were further analyzed by the SNK test (SAS Institute, 1985).

7.2.3. Results and Discussion

The effects of feeding oil seeds on the quality of egg albumen were examined by means of measuring Haugh units of eggs during the 6-wk storage period (Table 7.4). A rapid decline of Haugh units was found for eggs from all dietary treatments during the first 2 wk of storage (P<.05). Haugh units of eggs from Hn-9 and Hn-3 diets did not change significantly over the next 4 wk of storage, but those of eggs from the Hn-6 and control diets continued to decrease from Week 2 to 4. Feeding Hn-9 to laying hens resulted in higher (P<.05) Haugh units than feeding Hn-6. Overall, feeding oil seeds did not adversely affect egg Haugh units during storage.

Yolk color is an important quality trait in influencing consumer acceptance (Hunton, 1987). In the present study, both diet and storage significantly (P<.05) affected yolk color as measured by comparison to the Roche Yolk Color Fan (Table 7.4). For all dietary treatments yolk color index increased steadily over the storage period. The exact cause for such an increase of yolk color is not known at this point. Feeding full-fat flax seed to laying hens resulted in darker yolks than other dietary regimes (P<.05). Although having no influence on the nutritive value of eggs, darker yolks might be more appealing to consumers of certain ethnic groups. Storage up to 6 wk at 4 C did not significantly change yolk fatty acid profiles (data not shown).

One of the most important quality parameters in determining consumer acceptance of any food item is the sensory characteristic. As indicated in Figure 7.4, studies at both the University and the Food Centre generated similar preference patterns. Eggs from hens fed the Hn-3 diet scored significantly lower in preference evaluation than others while those from the sunflower seed diets were not affected when compared with the control. Exactly 36% of the evaluations in both studies detected a fishy or fish-product related flavor, such as "cod liver oil" and "tuna flavor", for eggs from the Hn-3 diet (Table 7.5).

When the hedonic scores of panellists who did not detect the fishy flavor were compared, no dietary treatment effect was found, indicating that the fishy flavor was the major cause of lower preference scores of eggs from the Hn-3 diet. The exact percentages of fishy flavor being reported in the two separate studies might be coincidental, but it revealed that approximately one third of the panellists were able to detect this off-flavor in eggs from the Hn-3 regime. Previously, Farrell and Gibson (1990) reported that eggs from hens fed diets containing fish oil, canola oil, or linseed (flax) oil were indistinguishable from those of the control. The discrepancy might be due to 1) dietary fat sources (whole seeds versus oils); and 2) methods of egg preparation. In this study, eggs were hard-cooked and one piece of the egg representing each dietary treatment was presented to a panellist. In the study of Farrell and Gibson (1990), six eggs from each treatment were blended with 30 mL of water and cooked in a 700 watt microwave oven on "high" for 3 min. The samples were stirred and reheated for a further 1 min and let stand for 2 min before serving.

The cause or causes of fishy flavors in eggs from laying hens fed flax seed remain to be determined. It was speculated that the fishy flavor could be due to the presence of 1) trimethylamine (TMA); 2) lipid oxidation products; and 3) the flax flavor per se. Fishy flavor has been described in eggs from hens fed either fishmeal (Wakeling, 1982) or apeseed meal (Hobson-Frohock et al., 1975), and TMA was the suspected substance in these eggs (Hobson-Frohock et al., 1973). Choline which was derived from sinapine in rapeseed meal was the precursor molecule for TMA (Hobson-Frohock et al., 1977). This sinapine -- choline -- TMA scheme, however, might not be the case in the Hn-3 fed laying hens in this experiment. Firstly, it has been repeatedly demonstrated that sinapine related fishy flavor was detected only in eggs from hen strains such as Rhode Island Reds but not from White Leghorns (Wakeling, 1982) which were used in the present study. Secondly, the sinapine or choline content of flax seeds was much lower than that of rapeseed.

The rancidity products of PUFA cause fishy flavor in some food products (Saxby, 1982). In the present study, eggs were stored at 4 C for 7 days before sensory evaluations were carried out. Therefore, there might be very little, if any, oxidation of PUFA occurred in yolks during the first wk of post harvest. The oxidative rancidity was more likely to occur in the Hn-3 diet during the preparation and feeding of the diet. These oxidation products might find a way into the yolk when the diet was fed to laying hens. As only a trace amount of lipid oxidation products are needed to cause fishy flavor in food products (Saxby, 1982), it might be possible that the fishy taint could be the result of rancidity of the Hn-3 diet.

The fishy flavor detected might also be the result of direct transfer of flavor compounds from flax seed into the yolk. One panellist from the University study who tasted fresh flax seed before described flax flavor rather than fishy flavor in a yolk sample from the Hn-3 diet. Therefore, it is possible that the so-called fishy flavor described by other panellists was actually the characteristic flavor of flax seed.

In brief, feeding full-fat flax and sunflower seeds to laying hens did not affect egg internal quality in terms of specific gravity and Haugh units. Yolk color was darkened by feeding flax seed. Furthermore, feeding flax seed in the present study resulted in a fishy flavor in some eggs. Questions on the extent of fishy eggs produced by hens fed the Hn-3 diet, the exact substance or substances that cause fishy flavors in these eggs, what components in Hn-3 diet are responsible for the off-flavors, and how to overcome these problems remain to be answered prior to any mass production and marketing of these fatty acid-modified eggs.

Table 7.1. Personal data of subjects before the experiment

Subject	Age	Height	Weight	BMI[1]
	y	cm	kg	
Group A				
1	25	175.3	56.8	18.5
2	19	185.4	77.2	22.4
3	19	177.8	77.2	24.4
4	28	176.5	74.9	24.0
5	32	172.7	65.8	22.1
6	18	177.8	70.4	22.3
7	18	180.3	72.6	22.3
8	22	175.3	84.0	27.3
9	22	170.2	68.1	23.5
10	19	190.5	84.0	23.1
11	21	180.3	65.8	20.2
x ± SD	22.1 ± 4.3	178.4 ± 5.5	72.4 ± 7.8	22.8 ± 2.2
Group B				
1	22	177.8	79.5	25.1
2	22	182.9	79.5	23.8
3	24	188.0	77.2	21.8
4	30	172.7	74.9	25.1
5	24	172.7	74.9	25.1
6	24	180.3	74.9	23.0
7	20	175.3	74.9	24.4
8	18	185.4	70.4	20.5
9	20	188.0	72.6	20.6
10	23	182.9	69.5	20.8
11	19	182.9	68.1	20.4
12	19	177.8	65.8	20.8
x ± SD	22.1 ± 3.1	180.6 ± 5.1	73.5 ± 4.2	22.6 ± 1.9

[1] Body mass index = Weight (kg) / (Height (m))^2.

Table 7.2. Major fatty acid composition of egg yolks[1]

Fatty acid	N-3 PUFA[2] enriched	Regular
	% of total fatty acid	
16:0	22.2 ± 0.2	25.9 ± 0.6
16:1	2.4 ± 0.1	3.3 ± 0.3
18:0	9.9 ± 0.3	8.6 ± 0.2
18:1	39.3 ± 0.9	44.6 ± 0.8
18:2 (n-6)	13.6 ± 1.1	10.9 ± 1.2
18:3 (n-3)	8.8 ± 0.5	1.4 ± 0.2
20:4 (n-6)	0.8 ± 0.1	1.3 ± 0.1
22:6 (n-3)	1.3 ± 0.1	0.8 ± 0.1
Σ n-6 PUFA	14.7	12.7
Σ n-3 PUFA	10.3	2.2
Σ(n-6)/Σ(n-3) ratio	1.4	5.7

[1] Mean ± SD. Three determinations of pooled yolk samples at the beginning of the trial.

[2] Polyunsaturated fatty acids.

Table 7.3. Major fatty acid composition of human plasma total lipids[1]

	Group A			Group B		
Fatty acid	Initial	Day 18	% change	Initial	Day 18	% change
	(% of total)			(% of total)		
16:0	23.1	22.9	- .8	22.0	22.6	+ 2.7
16:1	2.0	1.9	- 5.1	1.9	2.1	+ 12.7
18:0	7.7	7.8	+ .4	7.5	7.9	+ 5.1
18:1	23.5	23.2	- 1.5	22.1	22.4	+ 1.7
18:2 (n-6)	27.9	28.1	+ .6	29.5	28.0	- 5.0
18:3 (n-3)	.9	1.6	+ 68.2	1.0	.9	- 10.8
20:4 (n-6)	6.5	6.7	+ 4.1	6.9	6.8	- 1.9
20:5 (n-3)	.7	.8	+ 9.4	.9	.9	- 3.5
22:6 (n-3)	1.2	1.6	+ 32.8	1.8	1.9	+ 8.1
Σ n-6 PUFA[2]	34.4	34.8	+ 1.2	36.4	34.8	- 4.3
Σ n-3 PUFA	2.8	4.0	+ 42.9	3.7	3.7	0
Σ(n-6)/Σ(n-3)	12.3	8.7	- 29.2	9.8	9.4	- 4.1

[1] Mean of 11 or 12 subjects for Group A or Group B, respectively.

[2] Polyunsaturated fatty acids.

Table 7.4. Effects of feeding full-fat flax and sunflower seeds and storage on Haugh units and yolk color index of eggs[1]

Storage (wk)	Laying hen diet[2]				Overall
	Hn-3	Hn-9	Hn-6	Control	
	Haugh unit				
0	$78.2^{a,x}$	$83.3^{a,x}$	$79.2^{a,x}$	$78.7^{a,x}$	79.8^{x}
2	$66.9^{a,y}$	$66.3^{a,y}$	$67.8^{a,y}$	$71.6^{a,y}$	68.2^{y}
4	$64.9^{a,y}$	$65.2^{a,y}$	$59.0^{b,z}$	$59.0^{b,z}$	62.3^{z}
6	$65.6^{ab,y}$	$64.8^{a,y}$	$58.6^{b,z}$	$60.4^{ab,z}$	62.0^{z}
Overall	68.9^{ab}	69.9^{a}	66.2^{b}	67.4^{ab}	
	Yolk color index				
0	$3.4^{a,z}$	$2.0^{c,z}$	$2.5^{b,y}$	$3.2^{a,y}$	2.8^{z}
2	$3.5^{a,z}$	$2.2^{d,z}$	$2.8^{c,y}$	$3.2^{b,y}$	2.9^{z}
4	$3.7^{a,y}$	$3.2^{b,y}$	$3.1^{b,x}$	$3.5^{a,x}$	3.4^{y}
6	$4.1^{a,x}$	$3.6^{bc,x}$	$3.3^{c,x}$	$3.6^{b,x}$	3.7^{x}
Overall	3.7^{a}	2.7^{d}	2.9^{c}	3.4^{b}	

$^{a-d}$ Means in a row with no common superscripts differ significantly ($P<.05$).

$^{x-z}$ Means in a column with no common superscripts differ significantly ($P<.05$).

[1] Data (n = 24) were analyzed by two-way ANOVA followed by Student-Newman-Keuls test to separate means of main effects (diet and storage). Means of experimental units (diet by storage) were analyzed using the PDIFF function of the SAS^{R} program (SAS Institute, 1985).

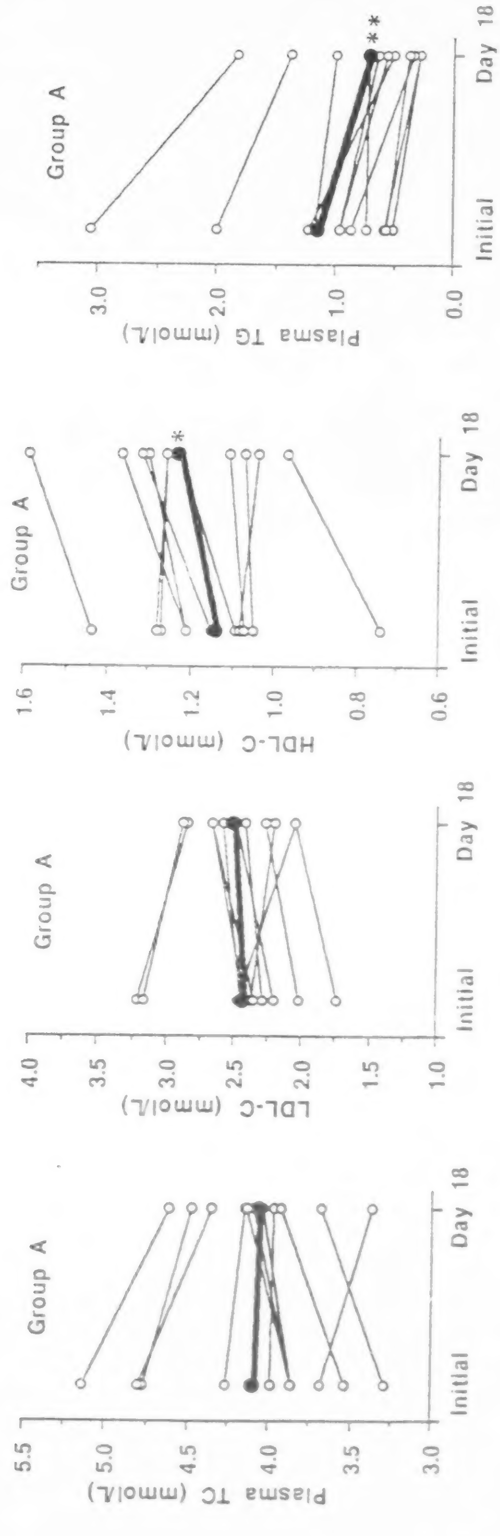
[2] The laying hen diets contained either 15% flax seed (Hn-3), 18% high oleic acid sunflower seed (Hn-9), 21% regular high linoleic acid sunflower seed (Hn-6), or 3% animal tallow (Control).

Table 7.5. Number and percentage of evaluations that reported fishy flavor in eggs from laying hens fed diets containing full-fat oil seeds

Study by		Laying hen diet[1]			
		Hn-3	Hn-9	Hn-6	Control
Food Center (n = 36)	Number	13	1	0	2
	Percentage	36	3	0	6
University (n = 28)	Times	10	0	0	0
	Percentage	36	0	0	0

[1] The laying hen diets contained either 15% flax seed (Hn-3), 18% high oleic acid sunflower seed (Hn-9), 21% regular high linoleic acid sunflower seed (Hn-6), or 3% animal tallow (Control).

Figure 7.1. Plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and plasma triglyceride levels in human subjects who consumed two n-3 PUFA-enriched (Group A, n=11) or regular eggs (Group B, n=12) a day with their habitual diets for a period of 18 days. Open circles represent individual subjects and the solid circles the mean of whole group. Means with * or ** are significantly different from initial values at 5% or 1% levels, respectively.



Group A
Group B
Plasma TC (mmol/L)
LDL-C (mmol/L)
HDL-C (mmol/L)
Plasma TG (mmol/L)
Initial
Day 18
5.5
5.0
4.5
4.0
3.5
3.0
6.0
2.5
2.0
1.5
1.0
1.6
1.4
1.2
0.8
0.6
0.0
*
* *

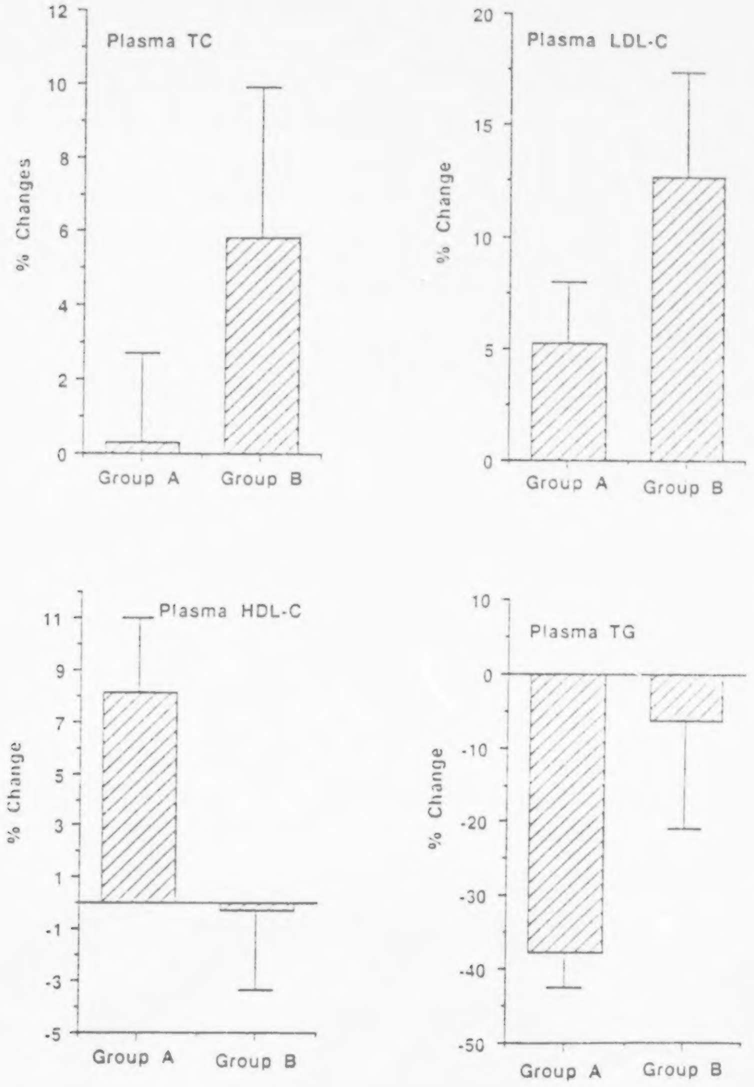


Figure 7.2. The percentage of changes in plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and plasma triglyceride (TG) levels in human subjects after consumption of two n-3 PUFA-enriched (Group A) or regular eggs (Group B) a day with their habitual diets for a period of 18 days. Data were presented as means ± SEM, n = 11 or 12 for Group A and Group B, respectively.

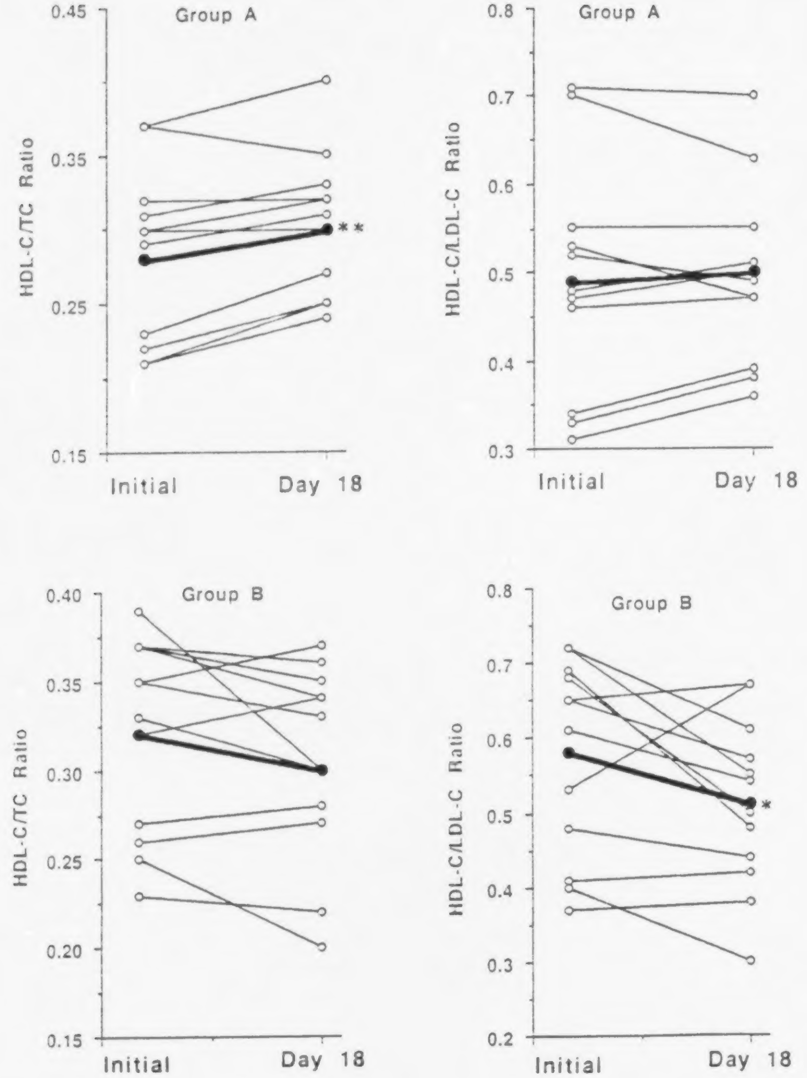


Figure 7.3. Changes of HDL-C/TC and HDL-C/LDL-C ratios in subjects after consumption of two n-3 PUFA-enriched eggs (Group A) or two regular eggs per day in addition to their habitual diet for a period of 18 days. Open circles represent individual subjects and the solid circles the mean of whole group. Means with * or ** are significantly different from initial values at 5% or 1% levels, respectively.

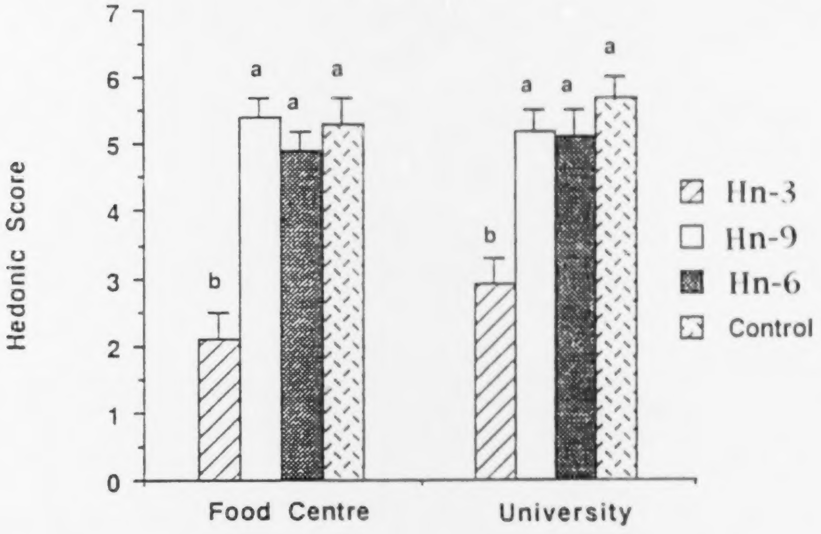


Figure 7.4. Effects of dietary full-fat oil seeds on the hedonic scores of eggs (x ± SEM, n = 36 and 28 for Food Centre study and University study, respectively). A hedonic score of 0 represents dislike extremely, and a 9 like extremely. Bars in the same graph with no common letters differ significantly ($P<.05$). The laying hen diets contained either 15% flax seed (Hn-3), 18% high oleic acid sunflower seed (Hn-9), 21% regular high linoleic acid sunflower seed (Hn-6), or 3% animal tallow (Control).

7.3. References

Ajuyah, A. O., K. H. Lee, R. T. Hardin, and J. S. Sim, 1991. Changes in the yield and in the fatty acid composition of whole carcass and selected meat portions of broiler chickens fed full-fat oil seeds. Poultry Sci. 70:2304-2314.

Applebaum-Bowden, D., S. M. Haffner, E. Hartsook E, K. H. Luk, J. J. Albers, and W. R. Hazzard, 1984. Down-regulation of the low-density lipoprotein receptor by dietary cholesterol. Am. J. Clin. Nutr. 39:360-367.

Burr, M. L., A. M. Fehily, and J. F. Gilbert, 1989. Effects of changes in fat, fish and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 2:757-761.

Canadian Consensus Conference on Cholesterol: Final Report, 1988. Can. Med. Assoc. J. 139(11):1-8.

Caston, L., and S. Leeson, 1990. Dietary flax and egg composition. Poultry Sci. 69:1617-1620.

Conner, S. L., and W. E. Conner, 1983. The importance of dietary cholesterol in coronary heart disease. Prev. Med. 12:115-123.

Connor, W. E., 1986. Hypolipidemic effects of dietary w-3 fatty acids in normal and hyperlipidemic humans: effectiveness and mechanisms. Pages 173-195 in: Health Effects of Polyunsaturated Fatty Acids in Seafoods. Simopoulos, A. P., R. R. Kifer, and R. E. Martin, eds. Academic Press, New York, NY.

Cruickshank, E. M., 1934. Studies in fat metabolism in the fowl. 1. The composition of the egg fat and depot fat of the fowl as affected by the ingestion of large amounts of different fats. Biochem. J. 28:965-977.

Dyerberg, J., and H. O. Bang, 1979. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 2:433-435.

Dyerberg, J., H. O. Bang, and E. Stofferson, 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet 2:117-119.

Eaton, S. B., and M. Konner, 1985. Paleolithic nutrition. A consideration of its nature and current implications. N. Engl. J. Med. 312:283-289.

Farrell, D. J., and R. A. Gibson, 1990. Manipulation of the composition of lipid in eggs and poultry meat. Pages 164-179 in: Proceedings of the Inaugural Massey Pig and Poultry Symposium. Massey University, Massey, New Zealand.

Folch, J., M. Lees, G. H. Sloan-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Garg, M. L., E. Sebokova, A. Wierzbicki, A. B. R. Thomson, M. T. Clandinin, 1988. Differential effects of dietary linoleic and α-linolenic acid on lipid metabolism in rat tissues. Lipids 23:847-852.

Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber, 1977. High density lipoprotein as a protective factor against coronary heart disease: The Framingham Study. Am. J. Med. 62:707-714.

Goldbourt, V., E. Holtzman, and H. N. Neufeld, 1985. Total and high density lipoprotein cholesterol in the serum and risk of mortality: evidence of a threshold effect. Br. Med. J. 290:1239-1243.

Grundy, M. S., E. Barrett-Connor, L. L.Rudel, T. Miettinen, and A. A. Spector, 1988. Workshop on the impact of dietary cholesterol on plasma lipoproteins and atherogenesis. Ateriosclerosis 8:95-101.

Hargis, P. S., M. E. van Elswky, and B. M. Hargis, 1991. Dietary modification of yolk lipid with Menhaden oil. Poultry Sci. 70:874-883.

Harris, W. S., 1989. Fish oils and plasma lipid and lipoprotein metabolism in humans: critical review. J. Lipid Res. 30:785-807.

Harris, W. S., W. E. Connor, N. Alam, and D. R. Illingworth, 1988. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. J. Lipid Res. 29:1451-1457.

Haugh, R. R., 1937. The Haugh unit for measuring egg quality. U. S. Egg Poult. Mag. 43:552-555, 572-573.

Health and Welfare Canada, 1990. Nutrition Recommendations. Canadian Government Publishing Centre, Ottawa, Canada.

Hobson-Frohock, A., G. R. Fenwick, R. K. Heanley, D. G. Land, and R. F. Curtis, 1977. Rapeseed meal and egg taint: association with sinapine. Br. Poult. Sci. 18:539-541.

Hobson-Frohock, A., G. R. Fenwick, D. G. Land, R. F. Curtis, and A. L. Gulliver, 1975. Rapeseed meal and egg taint. Br. Poult. Sci. 16:210-222.

Hobson-Frohock, A., D. G. Land, M. M. Griffiths, and R. F. Curtis, 1973. Egg taints: association with trimethylamine. Nature 243:304-305.

Hunton, P., 1987. Laboratory evaluations of egg quality. Pages 87-102 in: Egg Quality -- Current Problems and Recent Advances. R. G. Wells and C. G. Belyavin, ed. Butterworths Co. Ltd. London, England.

Jiang, Z., and J. S. Sim, 1992. Effects of dietary n-3 PUFA-enriched chicken eggs on plasma and tissue cholesterol and fatty acid composition of rats. Lipids (in press).

Jiang, Z., D. U. Ahn, AND J. S. Sim, 1991. Effects of feeding flax and two types on sunflower seeds on fatty acid composition of yolk lipid classes. Poultry Sci.70:2467-2475.

Karmali, R. A., J. March, and C. Fuchs, 1984. Effect of omega-3 fatty acids on growth of rat mammary tumor. J. Natl. Cancer Inst. 73:457-461.

Keys, A., 1984. Serum-cholesterol response to dietary cholesterol. Am. J Clin. Nutr. 40:351-359.

Kinsella, J. E., B. Lokesh, and R. A. Stone, 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52:1-28.

Knapp, H. R., 1989. Omega-3 fatty acids, endogenous prostaglandins and blood pressure regulation in humans. Nutr. Rev. 47:301-313.

Koehler, H. H., and M. Jacobson, 1966. Flavor of egg yolk: fractionation and profile analysis. Poultry Sci. 45:1371-1380.

Kritchevsky, D., S. A. Tepper, and D. M. Klurfeld, 1991. Influence of flaxseed on serum and liver lipids in rats. J. Nutr. Biochem. 2:133-134.

Kromhout, D., E. B. Bosschieter, and C. deL Coulander, 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312:1205-1209.

Leaf, A., and P. C. Weber, 1987. A new era for science in nutrition. Am. J. Clin. Nutr. 45 (suppl):1048-1053.

Masse, J., 1991. Effects of fish-oil ingestion on cardiovascular risk factors. Am. J. Clin. Nutr.54:610-611.

Metcalfe, L. D., A. A. Schmitz, and J. B. Pelka, 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem. 33:363-364.

Naber, E. C., 1983. Nutrient and drug effects on cholesterol metabolism in the laying hen. Fed. Proc. 42:2486-2493.

National Research Council (U.S), 1988. Committtee on Technological Options to Improve the Nutritional Attributes of Animal Products. Pages 18-44 in: Designing Foods: Animal Product Options in the Market. National Academy Press, Washington, D. C..

Nestel, P. J., 1986. Fish oil attenuates the cholesterol induced rise in lipoprotein cholesterol. Am. J. Clin. Nutr. 43:752-757.

Neuringer, M., and W. E. Connor, 1986. N-3 fatty acids in the brain and retina: evidence for their essentiality. Nutr. Rev. 44:285-294.

Noble, R. C., 1987. Egg lipids. Pages 159-177 in: Egg Quality -- Current Problems and Recent Advances. R. G. Wells and C. G. Belyavin, ed. Butterworths Co. Ltd. London, England.

Nwokolo, E., Z. Jiang, and J. S. Sim, 1988. Fatty acid composition and cholesterol contents of egg and tissues and serum lipoproteins of laying hens fed full-fat canola seed. Poultry Sci. 67(Suppl. 1):129.(Abstr.)

Oh, S. Y., J. Ryue, C. H. Hsieh, and D. E. Bell, 1991. Eggs enriched in w-3 fatty acids and alterations in lipid concentrations in plasma and lipoproteins and in blood pressure. Am. J. Clin. Nutr. 54:689-695.

Pyorala, K., 1987. Dietary cholesterol in relation to plasma cholesterol and coronary heart disease. Am. J. Clin. Nutr. 45:1176-1184.

Sanders, T. A. B., and F. Roshannai, 1983. The influence of different types of omega-3 polyunsaturated on blood lipids and platelet function in healthy volunteers. Clin. Sci. 64:91-96.

SAS Institute, 1985. SAS^R/STAT Guide for Personal Computers, 6th ed. SAS Institute, Inc., Cary, NC.

Saxby, M. J., 1982. Taints and off flavors in foods. Pages 439-457 in: Food Flavors. Part A. Introduction. I. D. Morton, and A. J. Macleod, ed. Elsevier Scientific Publishing Company, Amsterdam, The Netherlands.

Saynor, R., D. Verel, and T. Gillot, 1984. The effects of MaxEPA on the serum lipids, platelets, bleeding time and GTN consumption. Br. J. Clin. Pract. 38:(suppl 31):1626.

Shrimpton, D. H., 1987. The nutritive value of eggs and their dietary significance. Pages 11-25 in: Egg Quality -- Current Problems and Recent Advances. Wells, R. G., and C. G. Belyavin, eds. Butterrworth & Co. Ltd, London.

Sim, J. S., 1990. Flax seed as a high energy/protein/omega-3 fatty acid feed ingredient for poultry. Pages 65-71 in: Proceedings of the 53rd Flax Institute of the United States, Fargo, ND.

Sim, J. S., S. O. Chung, P. A. Borgersen, Z. Jiang, and W. P. Chorney, 1991. Cholesterol and fatty acid compositions of commercially produced Alberta eggs (A provincial-wide survey study). Pages 20-21 in: 70th Feeders' Day Report, University of Alberta.

Sim, J. S., Z. Jiang, and E. N. Nwokolo, 1988. Barley and full-fat canola seed (Barlcan) in poultry feeds. Poultry Sci. 67 (suppl. 1):156 (abstr).

Simopoulos, A.P., 1990. Genetics and nutrition: or what your genes can tell you about nutrition. Pages 25-34 in: Genetic Variation and Nutrition. Simopoulos A. P., and B. Childs, eds. World Rev. Nutr. Diet.

Simopoulos, A.P., 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54:438-463.

Singer, P., I. Berger, M. Wirth, W. Godicke, W. Jaeger, and S. Voight, 1986. Slow desaturation and elongation of linoleic and linolenic acid as a rationale of eicosapentaenoic acid-rich diet to lower blood pressure and serum lipids in norma, hypertensive and hyperlipidemic subjects. Prostagl. Leuko. Med. 24:173-193.

Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill Book Company, New York, NY.

US Department of Health and Human Services, 1988. The Surgeon General's report on nutrition and health. US Government Printing Office, Washington, D. C..

von Losonczy, T. O., A. Ruiter, H. C. Bronsgeest-Schoute, C. M. van Gent, and R. J. J. Hermus, 1978. The effect of a fish diet on serum lipids in healthy human subjects. Am. J. Clin. Nutr. 31:1340-1346.

Von Shacky, C., S. Fischer, and P. C. Weber, 1985. Long term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function and eicosanoid formation in humans. J. Clin. Invest. 76:1626-1631.

Vuilleumier, J. P., 1969. The 'Roche Yolk Color Fan'. An instrument for measuring yolk color. Poultry Sci. 48:767-779.

Wakeling, 1982. A fish taint in eggs: interaction between fishmeal diets and strain of birds. Br. Poult. Sci. 23:89-93.

Yu, M. M., and J. S. Sim, 1987. Biological incorporation of n-3 polyunsaturated fatty acids into chicken eggs. Poultry Sci. 66(Suppl. 1):195.(Abstr.)

Chapter 8. General Discussion

Despite tremendous research effort, the role of dietary cholesterol and plasma and lipoprotein cholesterol levels in coronary heart disease and atherogenesis remains uncertain (Stehbens, 1989; Steinberg, 1989). Meanwhile, the cholesterol content of chicken eggs, the richest source of dietary cholesterol, has received much attention. During the past several years, claims of "low cholesterol eggs" frequently appeared in the popular press and disappeared even faster. At the same time, the "official" cholesterol content for eggs reported by USDA decreased from 274 mg cholesterol per large egg in 1976 to 213 in 1989 (USDA, 1976, 1989). This prompted me to examine the true value of egg cholesterol content and the possible biological factors affecting it. I found that the conventional chemical colorimetric method overestimated egg cholesterol content, which might explain the higher values in the previous USDA report (USDA, 1976). The cholesterol concentration of egg yolk did not vary significantly among eggs, but the size of egg yolks did. Consequently, the cholesterol content of an egg depends on the size of the yolk which, in turn, is associated with egg size. Small eggs have small yolks and less cholesterol. It is misleading to market small eggs as "low cholesterol eggs", because they contain less nutrients too.

Although it is the richest source of dietary cholesterol, egg yolk is by no means made of only cholesterol. In fact, more than 60% of yolk dry matter is lipids (Noble, 1987). It is known that the type of dietary lipids affects cholesterol metabolism. A preliminary study comparing the dietary effects of whole yolk powder and crystalline cholesterol also suggested that yolk components other than cholesterol per se might contribute to the cholesterolemic properties of yolks (Jiang and Sim, 1991). Subsequent studies on the cholesterolemic properties of two major yolk components, namely, yolk neutral oil and yolk phospholipids, showed that egg yolk neutral oil was not as cholesterolemic as saturated coconut oil, and dietary egg lecithin specifically increased plasma high-density lipoprotein cholesterol levels. These results might explain the often-observed elevation of plasma HDL cholesterol levels in studies using a large number of whole eggs or egg yolks as the source of dietary cholesterol. The favorable dietary effects of egg phopholipids might also be useful for the egg industry which is attempting to diversify egg products.

The second part of this thesis explored the possibility of altering cholesterolemic properties of eggs by modifying the fatty acid composition of yolk lipids. In the past decade, interest has grown in the ameliorative and preventive effects of n-3 polyunsaturated fatty acids (PUFA) against cardiovascular diseases (Kinsella et al., 1990; Simopoulos, 1991). This thesis demonstrated that 1) it was feasible to enrich chicken eggs with n-3 PUFA through dietary manipulation; and 2) fatty acid modification of yolk lipids reduced the cholesterolemic properties of the egg in both experimental animals and humans. This technology can be applied to meat and other food animal products. But is it of importance for the food animal industry to modify the fatty acid composition of products? In my opinion, it is not only important but necessary for the food industry to provide consumers with products which are rich in n-3 PUFA relative to n-6 PUFA. Several lines of evidence suggested that our ancestors' diets were rich in n-3 PUFA, and low in total fats and n-6/n-3 ratio (Simopoulos, 1991). The drastic deviation from our traditional diets occurred only after the industrial revolution at the end of the last century. Today's food items, including meat, egg, milk, and other dairy products, are poor in n-3 PUFA and excessive in n-6 PUFA. Hydrogenation, a common practice in modern food industry, has further tilted the already imbalanced fatty acid composition of many food products by preferentially destroying n-3 PUFA (Simopoulos, 1991). This dietary imbalance results in an accumulation of n-6 over n-3 PUFA in tissue phospholipids, which, in turn, causes an excessive production of eicosanoids from the n-6 arachidonic acid (AA) (Kinsella et al., 1990). This may result in hyperresponsiveness and, if chronic, may gradually initiate or exacerbate several pathophysiological conditions (Kinsella et al., 1991). It is within this context that the current imbalance of n-6/n-3 PUFA in many food items poses undesirable impacts on consumers' health.

This thesis explored a practical technique to rectify this imbalance. The technique will not only benefit health conscious consumers, but will also bring enormous economic returns and incentives to the animal industry. N-3 fatty acids need not be provided as expensive capsulated oil concentrates but can be incorportated into some premium quality foods such as eggs, meat, milk, and other dairy products. It is a much safer way to provide these highly unsaturated fatty acids to consumers, as lipids in animal products are naturally protected from auto-oxidation when compared with oil concentrates. The long-tainted image of animal products can be improved, and a new era for animal industry may be on its way.

8.1. References

Jiang, Z., and J. S. Sim, 1991. Effects of feeding egg yolk powder on the plasma and tissue cholesterol levels in rats. Poultry Sci. 70:401-403.

Kinsella, J. E., B. Lokesh, and R. A. Stone, 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52:1-28.

Noble, R. C., 1987. Egg lipids. Page 159-177 in: Egg Quality - Current Problems and Recent Advances. R. G. Wells and C. G. Belyavin, ed. Butterworth & Co. Ltd, London.

Simopoulos, A. P., 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54:438-63.

Stehbens, W. E., 1989. The controversial role of dietary cholesterol and hypercholesterolemia in coronary heart disease and atherogenesis. Pathology 21:213-221.

Steinberg, D., 1989. The cholesterol controversy is over. Why did it take so long? Circulation 80:1070-1078.

USDA, 1976. Composition of Foods, Dairy and Egg Products, Raw-Processed-Prepared. USDA Agriculture Handbook 8-1. Washington, D. C..

USDA, 1989. Composition of Foods, Dairy and Egg Products, Raw-Processed-Prepared. USDA Agriculture Handbook 8-1. Washington, D. C..